The *Wolbachia* pandemic among arthropods: interspecies transmission and mutualistic effects

D I S S E R T A T I O N

zur Erlangung des akademischen Grades

d o c t o r r e r u m n a t u r a l i u m

(Dr. rer. nat.)
im Fach Biologie

eingereicht an der
Lebenswissenschaftlichen Fakultät
der Humboldt-Universität zu Berlin

von
Dipl.-Biol. Roman Zug

Präsidentin der Humboldt-Universität zu Berlin
Prof. Dr.-Ing. Dr. Sabine Kunst

Dekan der Lebenswissenschaftlichen Fakultät
Prof. Dr. Bernhard Grimm

Gutachter

1. Prof. Dr. Peter Hammerstein
2. Prof. Dr. Gregory D. Hurst
3. Prof. Dr. John H. Werren

eingereicht am
24.03.2017

Tag der mündlichen Prüfung
07.12.2017

Abstract

Endosymbiotic bacteria of the genus *Wolbachia* are extremely widespread among arthropod species. Their predominant mode of transmission is maternal inheritance, that is, through the eggs of infected females (vertical transmission). They are also able to move between unrelated hosts, even between different species (horizontal transmission). As a consequence of maternal inheritance, *Wolbachia* are selected to increase transmission through females at the expense of males. They achieve that by selfishly interfering with host reproduction in a number of intriguing ways. In addition to this reproductive parasitism, it can also pay for the symbionts to evolve traits that directly increase the fitness of infected females. While the importance of faithful vertical transmission and reproductive parasitism for *Wolbachia*'s success is undisputed, in this thesis, we analyze the role of horizontal transmission and mutualistic effects in the *Wolbachia* pandemic among arthropods.

First, we derive an estimate of the number of arthropod species that are infected with *Wolbachia*. Our estimate is based on more appropriate data than those used in earlier analyses. We find that *Wolbachia* are by far the most common reproductive parasites, thriving in millions of species. In order to explain this striking distribution, we develop a model of *Wolbachia* horizontal transmission between species, building on epidemiological theory and network theory. Our model is able to reproduce the high incidence levels commonly found in arthropod communities. In particular, our findings point to the importance of horizontal transmission over large phylogenetic distances. Given that successful horizontal transmission is likely to be facilitated by symbiont-induced host benefits, we then perform a comprehensive review of *Wolbachia*–arthropod mutualisms. One focus lies on symbiont-associated interference with pathogens. We show that the evidence of *Wolbachia*-induced host protection in nature is limited so far, but that, overall, host benefits associated with infection occur in diverse contexts. By means of a population genetic model, we then analyze the influence of host benefits on the infection dynamics of *Wolbachia*. Our findings show that the product of vertical transmission efficiency and relative fitness of an infected female is crucial for a symbiont's invasion success. This also holds for *Wolbachia* double infections, for which we derive invasion conditions and equilibrium frequencies for the first time. Our results corroborate that host benefits substantially facilitate invasion of *Wolbachia* into novel hosts. Finally, we examine the interactions between *Wolbachia* infection and the host immune system, with a focus on reactive oxygen species, a main

component of the arthropod immune response. We propose a hypothesis that explains differential immune responses in novel and coevolved associations. Taken together, the findings presented in this thesis argue for a significant involvement of horizontal transmission and mutualistic effects in the *Wolbachia* pandemic among arthropods.

Zusammenfassung

Endosymbiontische Bakterien der Gattung *Wolbachia* sind in Arthropodenarten extrem weit verbreitet. Sie werden überwiegend durch maternale Vererbung übertragen, also über die Eier infizierter Weibchen (vertikale Transmission), können aber auch zwischen unverwandten Wirten übertragen werden, ja sogar von Art zu Art (horizontale Transmission). Aufgrund ihrer maternalen Vererbung stehen *Wolbachien* unter Selektionsdruck, die Transmission über Weibchen auf Kosten von Männchen zu erhöhen. Dies gelingt ihnen, indem sie die Reproduktion ihrer Wirte eigennützig und auf verblüffende Arten beeinträchtigen. Zusätzlich zu diesem reproduktiven Parasitismus kann es sich für die Symbionten auch lohnen, die Fitness infizierter Weibchen direkt zu erhöhen. Während die Bedeutung von vertikaler Transmission und reproduktivem Parasitismus für den Erfolg von *Wolbachia* unbestritten ist, möchten wir in dieser Arbeit untersuchen, welche Rolle horizontale Transmission und mutualistische Effekte bei der *Wolbachien*-Pandemie unter den Arthropoden spielen.

Zunächst leiten wir eine Schätzung der Anzahl der Arthropodenarten her, die mit *Wolbachien* infiziert sind. Unserer Schätzung liegen geeignetere Daten zugrunde als solche, die in früheren Analysen verwendet wurden. Wir stellen fest, dass *Wolbachien* die mit Abstand häufigsten aller Reproduktionsparasiten sind und in Millionen von Arten vorkommen. Um diese erstaunliche Verbreitung zu verstehen, entwickeln wir ein Modell zur horizontalen zwischenartlichen Transmission von *Wolbachia*, das auf epidemiologischer und Netzwerk-Theorie aufbaut. Mit unserem Modell können wir das hohe Vorkommen nachvollziehen, das man gewöhnlich in Arthropodengemeinschaften findet. Insbesondere weisen unsere Ergebnisse auf die Bedeutung von horizontaler Transmission über große phylogenetische Distanzen hin. Da eine erfolgreiche horizontale Transmission wahrscheinlich dadurch begünstigt wird, dass die Symbionten ihrem Wirt einen Fitnessvorteil verschaffen, legen wir anschließend eine umfassende kritische Betrachtung von Mutualismen zwischen *Wolbachien* und Arthropoden vor. Dabei liegt ein Schwerpunkt auf der von den Symbionten ausgehenden Beeinträchtigung von Pathogenen. Wir weisen nach, dass die Belege für natürlich vorkommenden *Wolbachia*-induzierten Schutz der Wirte vor Pathogenen bisher noch begrenzt sind; insgesamt hingegen findet man Fitnessvorteile, die mit einer *Wolbachien*-Infektion einhergehen, in zahlreichen Zusammenhängen. Mithilfe eines populationsgenetischen Modells untersuchen wir danach den Einfluss von Fitnessvorteilen für den Wirt auf die Infektionsdynamik von *Wolbachia*. Unsere Resultate zeigen, dass das Produkt aus

vertikaler Transmissionsrate und relativer Fitness eines infizierten Weibchens entscheidend für den Invasionserfolg der Symbionten ist. Das gilt ebenso für *Wolbachia*-Doppelinfektionen, für die wir erstmalig Invasionsbedingungen und Gleichgewichtsfrequenzen herleiten. Unsere Ergebnisse bestätigen, dass Fitnessvorteile für den Wirt die Invasion von *Wolbachien* in neue Wirte erheblich erleichtern. Schließlich untersuchen wir die Wechselwirkungen zwischen einer *Wolbachien*-Infektion und dem Immunsystem des Wirtes, wobei ein Schwerpunkt auf reaktiven Sauerstoffspezies liegt, einem Hauptbestandteil der Immunantwort von Arthropoden. Wir schlagen eine Hypothese vor, die unterschiedliche Immunantworten in neuen und ko-evolvierten Assoziationen erklären kann. Zusammengefasst sprechen die Ergebnisse dieser Arbeit für einen wesentlichen Anteil von horizontaler Transmission und mutualistischen Effekten an der *Wolbachien*-Pandemie in Arthropoden.

Contents

Prologue

In August 2016, Rio de Janeiro played host to the Games of the XXXI Olympiad, thus becoming the first South American city to host the Summer Olympics. The lead-up to these Games was marked by several bad headlines, including a pervasive doping scandal and widespread human rights violations against locals. Both issues, however, were overshadowed by worldwide health concerns regarding the Zika virus epidemic that had been spreading in Brazil for quite some time. In February 2016, the World Health Organization (WHO) had declared the outbreak a Public Health Emergency of International Concern because Zika infections can cause fetal microcephaly and other birth defects (Rasmussen *et al.*, 2016). With no treatment or vaccine at hand, the most effective protective strategy is to control the Zika vector, *Aedes aegypti* mosquitoes. However, conventional mosquito control strategies involving insecticides or the reduction of larval breeding sites have proven to be largely inefficient, urging the need for novel mosquito control methods (Yakob and Walker, 2016). Indeed, an unconventional approach was quickly found. Even before the opening of the Games in Rio, two studies showed that a certain type of bacteria called *Wolbachia* is able to block Zika transmission by *Ae. aegypti* (Aliota *et al.*, 2016; Dutra *et al.*, 2016), and a large-scale release of *Wolbachia*-infected mosquitoes in South America is currently underway (Callaway, 2016).

While the interested public probably first learned of *Wolbachia* in the context of such novel biological control strategies (if it did at all), biologists might have heard of the bacteria primarily because of other pecularities. First, *Wolbachia* are considered the most common bacterial infection in the animal world, infecting a vast number of insects and other invertebrates around the globe. Whereas the WHO announced the end of the Zika epidemic in November 2016, the *Wolbachia* pandemic is here to stay. Moreover, these symbionts fascinate evolutionary biologists because they manipulate the reproduction of their hosts in bizarre ways to enhance their own transmission, which in itself is remarkable: *Wolbachia* are inherited during host reproduction, but, importantly, by females only. Hence, manipulations that increase transmission through females will be favored by natural selection. Such interference with host reproduction can have cut-throat effects, for

example killing all male offspring to the benefit of female offspring. Accordingly, *Wolbachia* have long been notorious for their 'reproductive parasitism'. It therefore came as a surprise when the antiviral effects of *Wolbachia* were first discovered (Hedges *et al.*, 2008; Teixeira *et al.*, 2008). In fact, these effects are neither restricted to Zika nor to *Ae. aegypti*, but rather operate against a broad range of viruses in a diverse range of insects. Just in case you feel a bit dizzy by now and start wondering how *Wolbachia* does all the tricks it does—you are not alone. The mechanisms underlying both the selfish manipulations and the antiviral effects remain largely unknown (Jiggins, 2016). Furthermore, given *Wolbachia*'s strict vertical transmission and their parasitic lifestyle, it seems more than fair to ask how they became so extremely widespread in the global arthropod community. If the bacteria are faithfully transmitted from mother to offspring, how can they ever infect novel host species? And in light of their harmful manipulations, shouldn't hosts be more effective in getting rid of the unbidden guests?

In order to answer these questions, we have to get to know two more tricks *Wolbachia* have up their sleeves. First, in addition to vertical transmission down the female germline, the symbionts are also able to move horizontally between unrelated hosts, even between different species. Just like Zika is transmitted to humans by a mosquito bite, *Wolbachia* can be transmitted from one insect species to another, say from a parasitoid wasp to its fly host when probing it for oviposition, where the wasps's ovipositor acts as a 'dirty needle'. Importantly, *Wolbachia* horizontal transmission events occur nowhere near as frequently as do vertical transmission events in an insect's lifetime (nor as infectious diseases occur in ours). Nevertheless, they play an important role on an evolutionary timescale, as we will see later on in this thesis. Second, besides their nasty reproductive manipulations, *Wolbachia* can also be benign to their hosts. After all, increasing the fitness of infected females directly enhances symbiont transmission through these females; hence, such mutualistic behavior will be selected as well. As you might have guessed, we have already encountered one instance of mutualism—protection against pathogens. However, antiviral effects as those found in Zika-infected mosquitoes might not be the best example of naturally occurring host protection (the reasons for this will also be discussed in this thesis). In any case, it will be crucial to elucidate the mechanisms underlying anti-pathogenic and other mutualistic effects of *Wolbachia*, not least to improve vector control measures.

In this thesis, we argue that these two features—horizontal transmission and mutualistic behavior—significantly contribute to the unparalleled success of *Wolbachia*. To this end, we analyze the effects of both characteristics

on the spread of *Wolbachia* by means of mathematical models. We combine the modeling with a thorough review of the full range of *Wolbachia*-induced mutualisms, including a critical assessment of anti-pathogenic effects. Our objective throughout the thesis is to gain a better understanding of the driving forces of the great *Wolbachia* pandemic among arthropods.

1 Introduction

1.1 Symbiont transmission modes and the outcomes of symbiosis

1.1.1 The conventional view

Symbiosis—the living together of unlike organisms—has long been acknowledged to be of fundamental importance in the history of life (De Bary, 1879; Douglas, 2010). Just take the most prominent example, which involves the origin of the mitochondria, the energy-harnessing organelles of eukaryotic cells: the latter are thought to have originated through primary endosymbiosis, in which bacterial symbionts hosted by archaeal cells were converted into what we now know as mitochondria—one of the major transitions in evolution (Maynard Smith and Szathmáry, 1997; Williams and Embley, 2015).

Endosymbiosis, in general, relates to any situation in which symbionts live within and in close association with their hosts. Bacterial endosymbionts are tremendously abundant among invertebrates, particularly among arthropods (Zchori-Fein and Bourtzis, 2011). Their effects on host fitness span the whole range from mutualism (beneficial) to parasitism (harmful). Symbiont transmission modes are likewise diverse, ranging from vertical (heritable) to horizontal (infectious). It is well recognized that the way in which symbionts are transmitted plays a crucial role in determining whether parasitism or mutualism will evolve. In the conventional view, horizontal transmission favors parasitism (Anderson and May, 1982), whereas vertically transmitted symbionts will evolve towards mutualism because their survival depends on that of their hosts (Fine, 1975; Ewald, 1987; Yamamura, 1993; Lipsitch *et al.*, 1995). For the same reason, vertical transmission is thought to select for stable coevolutionary relationships between symbiont and host. This is nicely illustrated by heritable bacterial symbionts such as *Buchnera* in aphids and *Wigglesworthia* in tsetse flies. These endosymbionts are housed in a specialized host organ, the bacteriome, and provide their hosts with essential nutrients, thereby directly increasing host fitness. In such cases, strict vertical transmission has inextricably linked the evolutionary fates of

symbiont and host and thus led to the evolution of mutualism. However, the notion that heritable symbionts evolve into mutualists is true only to some extent. Actually, vertical transmission by no means guarantees that symbionts increase the fitness of their hosts, and bacteria of the genus *Wolbachia* are the best example of that. To see why this is so, let us shortly delve into the subject of genetic conflict.

1.1.2 Selfish genetic elements, genetic conflict, and reproductive parasitism

Some genes (or larger genetic elements) within an individual's genome act to further their own evolutionary interests at the expense of the individual as a whole, which puts these elements into conflict with the rest of the genome. Such selfish genetic elements (including, among others, transposable elements, B chromosomes, and meiotic drivers) and the intragenomic conflict they create have far-reaching evolutionary consequences (Hurst *et al.*, 1996; Burt and Trivers, 2006; Werren, 2011). A particular type of genetic conflict arises between nuclear and cytoplasmic elements, deriving from the difference in their inheritance patterns (Cosmides and Tooby, 1981). While nuclear genes are typically inherited through both sexes, cytoplasmic elements, such as mitochondria, chloroplasts, and most heritable endosymbionts, are generally transmitted maternally only, that is, through the cytoplasm of the egg (sperm cells contain almost no cytoplasm). This results in a cytonuclear conflict, in particular over sex determination and sex ratios (Werren and Beukeboom, 1998). Autosomal nuclear genes are generally selected to produce a balanced sex ratio (Düsing, 1883; Fisher, 1930). By contrast, cytoplasmically inherited elements favor strongly female-biased sex ratios. In fact, when in male hosts, they are stuck in reproductive 'dead ends' and hence under strong selection to manipulate host reproduction in ways that increase transmission through females at the expense of males. That is exactly the strategy of 'reproductive parasites' such as *Wolbachia*. Reproductive parasitism thus represents an alternative to the strategy used by mutualistic symbionts (which is to directly increase the fitness of infected females). Actually, reproductive parasites can even afford to decrease host fitness, despite being transmitted predominantly vertically. This alternative has often been neglected in the face of the long-lasting notion that vertical transmission necessarily selects for stable mutualistic associations (Werren and O'Neill, 1997).

1.2 *Wolbachia*, the masters of reproductive parasitism

Intracellular bacteria of the genus *Wolbachia* are common heritable symbionts of arthropods and nematodes. Nematode-associated *Wolbachia* are generally assumed to be essential for host reproduction and survival and to have coevolved with their hosts on long time scales, although the patterns of coevolution may be more complex than previously thought (Lefoulon *et al.*, 2016). In any case, *Wolbachia*'s 'signature feature', reproductive parasitism, has never been found in nematode hosts, and in this thesis, we will focus on *Wolbachia*–arthropod relationships.

In this section, we give a concise overview of the biology of *Wolbachia*, with a focus on aspects that matters most to our subject.[1] We start by sketching the tremendous success of *Wolbachia*, in terms of the number and diversity of infected arthropod host species. Then, we discuss those issues commonly considered to be responsible for this success: efficient transmission through the female germline and manipulation of host reproduction. In doing so, we also outline major *Wolbachia*–host interactions and their biological basis. We then give an overview of the phylogeny of *Wolbachia*, highlighting the well-known fact that vertical transmission alone is insufficient to account for *Wolbachia*'s vast distribution among arthropods. We therefore outline important features of horizontal transmission of the symbiont between different host species. Lastly, we address another issue that is likely to contribute to *Wolbachia*'s remarkable success: beneficial effects of infection.

1.2.1 Distribution: a true success story

Wolbachia were discovered almost a century ago in the reproductive tissue of the mosquito *Culex pipiens* (Hertig and Wolbach, 1924), and the type species *Wolbachia pipientis* was formally described a few years later (Hertig, 1936). Long thought of as rare and inconsequential, *Wolbachia* are now known to infect an astonishing variety of arthropods, probably making them the most common endosymbionts in the world. Most notably, they are found in species from all major insect orders, in particular the most species-rich groups Coleoptera (beetles), Diptera (flies), Hymenoptera (ants, bees, wasps, and sawflies), and Lepidoptera (moths and butterflies). Besides

[1] For aspects of *Wolbachia* biology that are beyond the scope of this thesis (e.g. *Wolbachia* genetics and genomics, or their role in host speciation), we refer the reader to excellent reviews (Serbus *et al.*, 2008; Werren *et al.*, 2008; Brucker and Bordenstein, 2012).

insects, *Wolbachia* infect spiders, scorpions, mites, ticks (all belonging to the subphylum Chelicerata), isopods, amphipods, ostracods, cirripeds, cladocerans, and copepods (all belonging to Crustacea),[2] and springtails (Hexapoda) (Baltanás *et al.*, 2007; Werren *et al.*, 2008; Cordaux *et al.*, 2012; Wiwatanaratanabutr, 2013). With the recent discovery of *Wolbachia* in the millipede *Hungarosoma bokori* (Myriapoda) (Mock *et al.*, 2016), the bacteria have been found in every arthropod subphylum. Although mostly searched for in terrestrial arthropods, *Wolbachia* also occur in aquatic hosts (Sontowski *et al.*, 2015). The species richness of terrestrial arthropods, and of insects in particular, is stunning. A recent multi-method approach estimated the mean number of terrestrial arthropod species to be 6.8 million, of which 5.5 million are estimated to be insect species (Stork *et al.*, 2015). The first statistical analysis of *Wolbachia* infection frequencies estimated that considerably more than half of all terrestrial arthropod species are infected (Hilgenböcker *et al.*, 2008). However, the authors of that analysis also pointed to some weaknesses of the underlying data. In Chapter 2, we present an estimate based on a more appropriate data set and find approximately 40% of all terrestrial arthropod species to be infected, which still is a remarkably high number.

1.2.2 Vertical transmission

Wolbachia are predominantly transmitted vertically, through the eggs of infected females, and faithful transmission to the next generation is considered to be a cornerstone of *Wolbachia*'s tremendous evolutionary success. Indeed, the efficiency of vertical transmission, although somewhat variable in the field, is commonly very high and frequently close to 100% (Hoffmann *et al.*, 1996; Jiggins *et al.*, 2002c). Efficient maternal transmission requires bacterial localization to the female germline. In the following, we briefly describe how *Wolbachia* achieve that localization.

Wolbachia are present in the female germline stem cells (GSCs). As GSCs divide, the bacteria are partitioned between the self-renewing stem cell and the differentiating cystoblast which gives rise to the oocyte (Serbus *et al.*, 2008). Recent evidence shows that *Wolbachia* are able to promote GSC self-renewal via secretion of an effector protein (Ote *et al.*, 2016). During oogenesis, the bacteria utilize the host cytoskeleton, particularly

[2] We are aware that arthropod systematics is an area of considerable debate, concerning for example the monophyly of crustaceans (Regier *et al.*, 2010). However, this dispute is of minor importance to our subject and does not impair the impressive abundance of *Wolbachia* among arthropod species.

microtubules and associated proteins (Ferree *et al.*, 2005; Serbus and Sullivan, 2007), and actin (Newton *et al.*, 2015), to ensure their localization at the posterior pole of the oocyte, which is the site of the future germline. Throughout late oogenesis and early embryogenesis, *Wolbachia* remain concentrated in the germ plasm at the posterior cortex (Hadfield and Axton, 1999; Serbus *et al.*, 2008). Germ plasm localization of *Wolbachia* has been observed in several insect species, suggesting that it is a successful strategy to ensure transmission to the next generation (Breeuwer and Werren, 1990; Zchori-Fein *et al.*, 1998; Veneti *et al.*, 2004).

An alternative strategy for *Wolbachia* to access the developing egg is through a mechanism called 'stem cell niche tropism'. Stem cells reside in a special microenvironment termed the stem cell niche. The *Drosophila* ovary contains two stem cell niches, the somatic stem cell niche (SSCN) and the germline stem cell niche (GSCN), supporting somatic stem cells (SSCs) and GSCs, respectively (Li and Xie, 2005). Interestingly, *Wolbachia* target and colonize both the *Drosophila* SSCN and the GSCN to reach germline cells, suggesting that stem cell niche tropism plays an important role in germline infection (Frydman *et al.*, 2006; Fast *et al.*, 2011). *Wolbachia* stem cell niche tropism has also been found in other insects (Hosokawa *et al.*, 2010; Sacchi *et al.*, 2010) and is probably an evolutionarily conserved mechanism for transmission to the germline (Toomey *et al.*, 2013).

Lastly, *Wolbachia* can reach the germline via association with the centrosomes in the early syncytial *Drosophila* embryo (Callaini *et al.*, 1994; Kose and Karr, 1995; Tram *et al.*, 2003). During the syncytial divisions, the bacteria undergo microtubule-dependent and cell-cycle-regulated movement between centrosomes, which results in an even distribution throughout the embryo (Albertson *et al.*, 2009). Consequently, a subset of centrosomes and associated *Wolbachia* end up in the posterior pole of the embyro, which ensures bacterial integration into prospective germline cells. However, this transmission strategy, if it is to be successful, requires a high *Wolbachia* titer in the embryo (Serbus *et al.*, 2008).

1.2.3 Reproductive parasitism

Around the same time Hertig and Wolbach (1924) discovered *Wolbachia* in the ovaries of *C. pipiens*, an entomologist in Fiji wondered why broods of the great eggfly, *Hypolimnas bolina*, contained only females (Simmonds, 1923). In subsequent work, he found this all-female trait to be transmitted by mothers only and concluded that the trait resulted from the death of sons. Since then, several other maternally inherited reproductive aberra-

tions have been discovered, including all-female broods in the woodlouse *Armadillidium vulgare*, but without differential mortality between the sexes (Vandel, 1941), incompatible crosses between certain strains of *C. pipiens* (Laven, 1951, 1956), and reversal to sexual reproduction in parthenogenetic *Trichogramma* wasps after antibiotic treatment (Stouthamer *et al.*, 1990). Today, these phenotypes are known as male-killing, feminization, cytoplasmic incompatibility, and parthenogenesis induction, respectively, and are collectively referred to as 'reproductive parasitism' (Hurst and Frost, 2015). It is also known by now that phenotypes of reproductive parasitism affect a broad range of arthropod hosts and are induced by a variety of maternally inherited microbes, including *Arsenophonus*, *Cardinium*, *Rickettsia*, *Spiroplasma*, and *Wolbachia* (Engelstädter and Hurst, 2009). Among those reproductive parasites, however, *Wolbachia* are special in that they are the only microbes known so far to induce all four phenotypes. Surely, *Wolbachia*'s mastery in reproductive parasitism is reflected in their unparalleled distribution in the animal world.

As discussed in Section 1.1.2, maternally inherited symbionts are under selection to increase the proportion of infected females. One way to achieve this is to distort the offspring sex ratio of infected mothers towards females. That is the rationale behind feminization and the induction of thelytokous parthenogenesis (which, effectively, is a form of feminization in haplodiploid hosts; see below): both phenotypes convert non-transmitting males into transmitting females, thus directly increasing symbiont fitness. The logic of another sex ratio distorting phenotype, male killing (MK), can be understood in terms of kin selection. MK is advantageous to symbionts when infected sisters of killed males benefit from their brothers' death through some form of fitness compensation. Hence, male-killing endosymbionts increase not their direct, but their indirect fitness. The most elaborate reproductive manipulation, cytoplasmic incompatibility (CI), does not distort sex ratios. Instead, CI-inducing symbionts exert a form of conditional sterility on their hosts: uninfected females suffer high offspring mortality when mating with infected males. By contrast, infected females can mate successfully with both infected and uninfected males. CI thus benefits infected females and favors the spread of symbionts through host populations. In the following, we present each reproductive manipulation phenotype in a little more detail.[3]

[3] In describing the mechanistic details of these phenotypes, we concentrate on those mechanisms known to be employed by *Wolbachia*. For more comprehensive reviews including other reproductive parasites, see for example Kageyama *et al.* (2012), Ma *et al.* (2014), and Hurst and Frost (2015).

Feminization

Conceptually, the conversion of genetic males into functional females is the most obvious manipulation for a maternally inherited symbiont that finds itself in a male host. *Wolbachia*-induced feminization has so far been found in the insect orders Hemiptera and Lepidoptera, in isopod crustaceans (Werren *et al.*, 2008), and possibly in a spider (Curry *et al.*, 2015). Feminization occurs through different mechanisms in different hosts and is not restricted to a specific sex determination system. In the isopod *A. vulgare*, genetic sex determination follows female heterogamety (ZZ males and ZW females). Male development is controlled by the androgenic hormone, which is produced by the androgenic gland. *Wolbachia* proliferate within the androgenic gland during early development, which prevents its differentiation and results in female development of ZZ individuals (Bouchon *et al.*, 2008). All-female production in the butterfly *Eurema mandarina* (former *Eurema hecabe*) was long considered to be due to the feminization of genetic males (ZZ) (Hiroki *et al.*, 2002; Narita *et al.*, 2007), but recent research suggests that things are more complicated. Since, unexpectedly, *E. mandarina* females seem to depend on their symbionts for proper development, we postpone a more detailed discussion to Chapter 4. In the male-heterogametic leafhopper *Zyginidia pullula*, *Wolbachia* disrupt the host genomic imprinting, with genetic males exhibiting a female-specific methylation pattern (Negri *et al.*, 2009). Finally, it is possible that, even in insects, *Wolbachia* induce feminization by interfering with hormonal pathways (Negri, 2012), although the role of hormones in insect sexual differentiation is under debate (Prakash and Monteiro, 2016).

Feminizing symbionts can have profound effects on the evolution of sex determination systems in their hosts (Cordaux *et al.*, 2011). An important evolutionary outcome of feminization in female-heterogametic (e.g. ZW) hosts is the elimination of the female sex chromosome (W). The reason is that feminized ZZ individuals produce functional females without transmitting the W chromosome. The population sex ratio is hence determined by the presence/absence of the feminizer—an example of cytoplasmic sex determination. In *A. vulgare*, genes have been found that prevent feminization by resisting *Wolbachia* transmission (Rigaud and Juchault, 1992). By increasing the proportion of males, these suppressor genes contribute to restore a more balanced population sex ratio and thus indirectly impact on sex determination. Chromosomal sex determination can be fully restored if a fragment of the *Wolbachia* genome that carries the feminization information (the '*f* element') is inserted into an autosome

of the host. This is exactly what has recently been reported in *A. vulgare*, where horizontal transfer of the *f* element has given birth to a new W sex chromosome (Leclercq *et al.*, 2016).

Induction of thelytokous parthenogenesis

Many insects, including all hymenopterans (ants, bees, and wasps), exhibit haplodiploid sex determination: unfertilized eggs develop into haploid males (a process termed arrhenotoky), while fertilized eggs develop into diploid females. Arrhenotoky thus constitutes a form of parthenogenesis (which is the production of viable offspring from unfertilized eggs). In many haplodiploid species, including hymenopterans, thrips, and mites (Werren *et al.*, 2008), *Wolbachia* are able to convert arrhenotokous parthenogenesis into thelytokous parthenogenesis, where unfertilized eggs develop into diploid females. Therefore, labeling this reproductive manipulation as 'parthenogenesis induction' is, strictly speaking, not correct (though more convenient). So far, this phenotype has been reliably documented only in haplodiploid species.

The simplest way for symbionts to induce thelytoky in hosts with haplodiploid sex determination is to cause diploidization of unfertilized eggs. Following the host's sex determination system, these diploidized eggs would then develop into females. In parasitoid wasps, *Wolbachia*-induced diploidization commonly occurs through a process called gamete duplication, where meiosis is normal, but diploidy is restored by disruption of the cell cycle during early embryonic development. In *Leptopilina clavipes* and several *Trichogramma* species, chromosomes fail to segregate in the first mitotic division (Stouthamer and Kazmer, 1994; Pannebakker *et al.*, 2004). In *Muscidifurax uniraptor*, the first division proceeds normally, but then the two cell nuclei fuse to produce a diploid female (Gottlieb *et al.*, 2002). A different mode than gamete duplication was observed in the mite *Bryobia praetiosa*, where *Wolbachia*-infected eggs presumably do not undergo meiosis, resulting in diploid gametes (Weeks and Breeuwer, 2001).

Diploidization has long been assumed to automatically lead to feminization in haplodiploid species. However, the occurrence of diploid males in the *Wolbachia*-infected thelytokous wasp *Asobara japonica* suggests that diploidization and feminization can be separate processes (Ma *et al.*, 2015). These findings support a two-step mechanism for endosymbiont-induced thelytoky, at least in *A. japonica*: diploidization of the unfertilized egg is followed by feminization, where each step relies on a specific symbiont density (Ma *et al.*, 2015). The frequency of this two-step mechanism among symbiont-induced thelytokous species needs to be clarified.

Wolbachia-induced thelytoky can have an interesting evolutionary implication in populations where infection has gone to fixation. In such all-female populations, the loss of sexual traits makes females dependent on their symbionts for daughter production. In Chapter 4, we will discuss this issue more extensively.

Male-killing

Ever since Simmonds (1923) discovered the male-killing phenotype in Fiji butterflies, the trait has been found in a plethora of host species. *Wolbachia*-induced male-killing[4] (MK) has so far been documented in the insect orders Coleoptera, Diptera, and Lepidoptera, and in a pseudoscorpion (Werren *et al.*, 2008). Although a sex-ratio distorting phenotype like feminization and thelytoky induction, the logic behind MK is different. By killing all male offspring of an infected female, the symbionts do not increase their transmission through that female directly. Rather, the adaptive rationale of MK can be explained by a kin selection argument: male death must benefit their surviving sisters who will pass to their offspring the clone-mates of the male-killer. The MK-associated benefit, termed 'fitness compensation', can come about in various ways: (i) reduced competition between siblings, (ii) reduced inbreeding and inbreeding depression, and/or (iii) resource reallocation, e.g. through sibling egg cannibalism (Hurst, 1991b).

In principle, MK could be achieved by targeting any male-specific feature that is essential for normal development. This might explain the diversity in mechanisms underlying symbiont-induced MK. In *Drosophila bifasciata*, *Wolbachia*-induced MK is associated with defective sperm chromatin remodeling (Riparbelli *et al.*, 2012). Sperm chromatin remodeling is one of the earliest processes in *Drosophila* fertilization and involves the removal of protamines and other sperm-specific proteins and subsequent *de novo* chromatin assembly using maternally provided histones (Loppin *et al.*, 2015). In *D. bifasciata*, defects in sperm chromatin remodeling are followed by abnormal mitotic spindle formation and chromosome condensation/segregation defects in male embryos (Riparbelli *et al.*, 2012). Interestingly, these defects are highly reminiscent of those observed in CI (see below), pointing to a similar mechanism underlying both phenotypes. It is unclear, however, why only

[4]Male-killing induced by *Wolbachia* and other bacterial endosymbionts is also called *early* male-killing (as it usually occurs early in development), to distinguish it from *late* male-killing, which is induced by microsporidia and RNA viruses at larval or pupal stages. Late male-killing rests upon a different adaptive logic and will not be considered further.

the chromatin of male embryos is affected, and which specific mechanisms are involved. *Wolbachia*-induced MK in the moths *Ostrinia furnacalis* and *O. scapulalis*, which exhibit female heterogamety (females: ZW; males: ZZ), is caused by a failure of dosage compensation, a process necessary to adjust the expression of Z-linked genes in males. In *O. furnacalis*, dosage compensation failure is due to repression of the *Masculinizer* (*Masc*) gene (Fukui *et al.*, 2015), and it is likely that the same mechanism underlies MK in *O. scapulalis* (Sugimoto *et al.*, 2015). Quite unusually, however, both moth species seem to have become dependent on their male-killer for proper female development. In Chapter 4, we will elaborate further on that dependence.

Suppression of *Wolbachia*-induced MK has been observed in different hosts (Hornett *et al.*, 2006; Jaenike, 2007; Vanthournout and Hendrickx, 2016). Selection for MK suppression is intense, as evidenced by the rapid spread of suppressor genes (Charlat *et al.*, 2007). Interestingly, however, suppression of the MK phenotype does not necessarily eliminate *Wolbachia*'s ability to induce MK. When *Wolbachia* that induce CI in their native host *Drosophila recens* were introgressed into *D. subquinaria*, they caused essentially complete MK in the novel host (Jaenike, 2007). These findings suggest that *Wolbachia* in *D. recens* have maintained their MK ability despite host resistance, and that they can induce two distinct phenotypes, CI and MK, again indicating a similar underlying molecular basis.

Cytoplasmic incompatibility

Cytoplasmic incompatibility (CI) is considered the most common reproductive manipulation, and at the same time, it is the only one that is not associated with sex ratio distortion. So far, *Wolbachia* and *Cardinium* are the only symbionts known to induce CI. *Wolbachia*-induced CI has been observed in the insect orders Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, in isopod crustaceans, and in mites (Werren *et al.*, 2008). CI is a reproductive incompatibility in matings between infected males and females that do not harbor the same symbiont strain. Offspring from those matings suffer mortality at early stages of their development. Unidirectional CI, the simplest form, usually occurs between infected males and uninfected females (the reciprocal cross is fully compatible), whereas bidirectional CI occurs when mating partners are infected with different strains (then both crosses are incompatible). In any case, infected females are compatible with both uninfected males and males harboring the same strain; uninfected females, by contrast, are compatible

only with uninfected males. Therefore, infected females have a selective advantage over uninfected females, which drives the spread of CI-inducing symbionts.

Despite intense empirical and theoretical efforts, the exact molecular mechanism of CI remains unknown (although most recent research marks a big step forward; see below). In *Drosophila*, the earliest observed defects associated with CI occur during sperm chromatin remodeling immediately after fertilization. Whereas protamine removal at the male pronucleus functions normally in CI crosses, subsequent deposition of histones H3.3 and H4 is abnormal, and paternal DNA replication seems to be impeded (Landmann *et al.*, 2009). These interphase defects in the male pronucleus could explain the chromosome condensation and segregation defects observed during the first mitotic division in CI embryos (Callaini *et al.*, 1997; Landmann *et al.*, 2009). Impaired H3.3 deposition is possibly due to decreased *Hira* expression in *Wolbachia*-infected *Drosophila* males; *Hira* encodes a chaperone of H3.3 (Zheng *et al.*, 2011a).

A hallmark of CI is the fact that the defects in the first zygotic division do not occur if the female is also infected. This phenomenon is usually conceptualized by a modification/rescue model: symbionts modify the sperm (the *mod* function), and the same strain must be present in the egg to rescue this modification (the *resc* function) (Werren, 1997). Interestingly, *mod* and *resc* are functionally independent, illustrated by the existence of the $mod^{-}resc^{+}$ phenotype which is unable to induce CI but capable of rescuing it (Poinsot *et al.*, 2003). Within the *mod*/*resc* conceptualization (which makes no assumption about the actual nature of the *mod* and *resc* factors), several more specific models have been proposed, namely the 'mistiming' (or 'slow motion') model, the 'lock and key' model, and the 'titration-restitution' model (Poinsot *et al.*, 2003; Serbus *et al.*, 2008). More recently, the 'mistiming' and the 'lock and key' model have been extended by means of sophisticated theoretical methods, drawing on formal logic and graph theory (Bossan *et al.*, 2011; Nor *et al.*, 2013). Still, we do not know yet how CI works.

Recently, Pontier and Schweisguth (2015) challenged the *mod*/*resc* concept by proposing that *Wolbachia*-induced CI is regulated by pheromone-based communication between male and female *Drosophila* pupae. However, in an attempt to reproduce these findings, Jacquet *et al.* (2017) failed to detect any influence of pupal communication on *Wolbachia*-mediated CI, both in *Drosophila* and *Nasonia*, questioning the pupal communication model for CI. Indeed, most recent evidence supports the modification/rescue model. LePage *et al.* (2017) found promising candidate genes in the genome

of the *Wolbachia* strain *w*Mel that induces CI in *D. melanogaster*. These two genes, named cytoplasmic incompatibility factor A (*cifA*) and B (*cifB*), are located in the prophage WO region of the *w*Mel genome. Dual *cifA*/*cifB* expression in transgenic, uninfected males crossed to uninfected females causes embryonic defects and lethality, essentially recapitulating CI. Notably, these defects are rescued in embryos from *w*Mel-infected females (LePage *et al.*, 2017). Moreover, the homologues of *cifA* and *cifB* were identified at the protein level in sperm from *Culex pipiens* mosquitoes infected with the *w*Pip strain that also induces CI (Beckmann and Fallon, 2013). The *w*Pip homologues, termed *cidA* and *cidB*, were shown to encode a deubiquitylating enzyme and its binding protein. *cidA* and *cidB* are part of a two-gene operon, similar to bacterial toxin–antidote systems. Again, in transgenic *Drosophila*, the *cidA*-*cidB* operon mimics CI (Beckmann *et al.*, 2017). By identifying bacterial genes responsible for CI, LePage *et al.* (2017) and Beckmann *et al.* (2017) have taken a big step towards the elucidation of its molecular mechanism.

A fundamental property of the infection dynamics of CI is that the drive associated with CI is positively frequency-dependent: the more infected males exist in a population, the more commonly uninfected females suffer from incompatible matings. This frequency-dependent selection results in the existence of a threshold infection frequency below which deterministic invasion of CI is not possible (at least with imperfect vertical transmission or infection-associated fitness costs) (Caspari and Watson, 1959; Fine, 1978; Hoffmann *et al.*, 1990). As we will see in Chapter 5, *Wolbachia*-induced fitness benefits can easily remove the invasion threshold.

1.2.4 Phylogeny: a telling lack of congruence

Wolbachia belong to the order Rickettsiales within the Alphaproteobacteria. The Rickettsiales are a diverse group of intracellular host-associated bacteria, comprising species with mutualistic, commensal and parasitic lifestyles. Intriguingly, this order most likely also includes the ancestor of mitochondria (Wang and Wu, 2015). In contrast to mitochondria, however, the widespread conflict between *Wolbachia* and (most of) their hosts suggests that these symbionts are a long way from undergoing a similar major transition.

Based on multiple genetic markers, *Wolbachia* are classified into major phylogenetic lineages termed supergroups, designated by capital letters (A, B, C, ...). New supergroups are still being discovered; the two most recently described supergroups are P and Q, which were found in quill

mites (Glowska *et al.*, 2015; Gerth, 2016). *Wolbachia* supergroups differ in their host distribution: strains of supergroups A and B infect a huge number of terrestrial arthropods, supergroup C and D strains are found in filarial nematodes, and other supergroups are known only from a small number of hosts restricted to specific (mainly arthropod) taxa. Supergroup F is as yet the only one to harbor strains from arthropods and nematodes. The phylogenetic relationships between supergroups are subject to extensive research, which is currently flourishing with a growing set of phylogenomic analyses (Comandatore *et al.*, 2013; Gerth *et al.*, 2014; Brown *et al.*, 2016). According to these analyses, the ubiquitous arthropod-associated *Wolbachia* strains all belong to a single monophyletic lineage consisting of supergroups A and B. The ability to adapt to a broad range of diverse host species seems to be restricted to that lineage and hence has a single evolutionary origin (Gerth *et al.*, 2014). Recently, the origin of that monophyletic lineage was estimated to be around 200 million years ago (Gerth and Bleidorn, 2016). Interestingly, this age coincides with the diversification of several major insect orders, including the 'megadiverse' Diptera, Hymenoptera, and Lepidoptera (Misof *et al.*, 2014). Insect radiation hence may have promoted the spread of *Wolbachia* by providing a plethora of novel host species (Gerth and Bleidorn, 2016).

Strikingly, fine-scale analyses of the phylogeny of *Wolbachia* and their arthropod hosts have repeatedly revealed extensive disagreement between both phylogenies, with identical *Wolbachia* strains infecting distantly related host species and closely related hosts harbouring different and distantly related symbiont strains (O'Neill *et al.*, 1992; Rousset *et al.*, 1992; Werren *et al.*, 1995b; Zhou *et al.*, 1998). More recent phylogenetic analyses based on multilocus sequence typing (MLST) (Baldo *et al.*, 2006) corroborate these findings for diverse host taxa, including flies (Sheeley and McAllister, 2009; Stahlhut *et al.*, 2010; Schuler *et al.*, 2013; Morrow *et al.*, 2014), bees (Gerth *et al.*, 2013), wasps (Raychoudhury *et al.*, 2009), ants (Frost *et al.*, 2010), beetles (Rodriguero *et al.*, 2010), butterflies (Salunke *et al.*, 2012; Ahmed *et al.*, 2016), and spiders (Baldo *et al.*, 2008). This lack of phylogenetic congruence between *Wolbachia* and their arthropod hosts clearly indicates that co-divergence is rare, and that *Wolbachia* horizontal transmission between distinct host species occurs frequently over evolutionary time.

1.2.5 Horizontal transmission

The clear evidence for common horizontal transmission of *Wolbachia* among arthropod species strongly suggests that interspecies transmission has contributed greatly to *Wolbachia*'s success (O'Neill *et al.*, 1992; Rousset *et al.*, 1992; Werren *et al.*, 1995b; Werren and Windsor, 2000). In Chapter 3, we present an epidemiological model of *Wolbachia* transmission between species that supports the view that horizontal transmission is required to explain the staggering abundance of arthropod species infected with *Wolbachia*. First, however, let us have a look at how such horizontal transmission might come about. In this respect, it is useful to distinguish three different stages in the process of horizontal transmission (also called filters) (Vavre *et al.*, 2003; Riegler *et al.*, 2004): (i) physical transfer from an infected host to a potential new host species (ecological filter); (ii) survival in the new host and infection of the germline (physiological filter); and (iii) spread through host populations (population filter). In the following, we briefly elaborate on these filters.

Physical transfer of *Wolbachia* requires close contact between donor and recipient host species. Several mechanisms and ecological contexts have been proposed, including parasitoids (Schilthuizen and Stouthamer, 1997; Vavre *et al.*, 1999a), social parasitism (Dedeine *et al.*, 2005a), common food substrates (Kittayapong *et al.*, 2003; Sintupachee *et al.*, 2006; Stahlhut *et al.*, 2010; Morrow *et al.*, 2014; Li *et al.*, 2016), transmission inside galls and figs (Rokas *et al.*, 2002; Haine and Cook, 2005; Yang *et al.*, 2013), phoresis (Covacin and Barker, 2007; Ahmed *et al.*, 2015), predation and cannibalism (Haine *et al.*, 2005; Le Clec'h *et al.*, 2013; Brown and Lloyd, 2015; Faria *et al.*, 2016), and via hemolymph, e.g. after injury (Rigaud and Juchault, 1995; Cordaux *et al.*, 2001).

After transfer to a novel host, *Wolbachia* must be able to survive in the new environment and reach the germline to establish efficient vertical transmission. To begin with, the bacteria need to cope with the native microbiota (Hughes *et al.*, 2014; Rossi *et al.*, 2015) and with the host immune response. We have devoted Chapter 6 to a more detailed discussion of the latter topic and here concentrate on the colonization of the germline. Immediately after host transfer, the bacteria are likely to be present only in somatic tissues (Frost *et al.*, 2014; Pietri *et al.*, 2016). The necessary soma-to-germline transmission usually requires the crossing of several tissues and cell membranes, including extracellular survival and direct cell-to-cell transmission (Pietri *et al.*, 2016). Indeed, *Wolbachia* injected into the abdominal cavity of *Drosophila* females were shown to migrate through

several tissues and infect the germline by entering through the somatic stem cell niche, suggesting that stem cell niche tropism plays a role in germline infection not only during vertical transmission (see above, Section 1.2.2), but also after horizontal transmission (Frydman *et al.*, 2006; Toomey *et al.*, 2013). Moreover, *Wolbachia* are able to survive in cell-free medium for up to one week, and in a leafcutter ant, they are abundantly found in the gut lumen, suggesting that they can survive extracellularly for some time (Rasgon *et al.*, 2006; Andersen *et al.*, 2012). To achieve cell-to-cell transfer, *Wolbachia* utilize the host actin cytoskeleton and clathrin/dynamin-dependent endocytotic pathways (Sheehan *et al.*, 2016; White *et al.*, 2017a). Cell exit and entry may also be facilitated by the fact that, within the host cell, *Wolbachia* reside in membraneous vesicles that have been found to be associated with the Golgi apparatus and the endoplasmic reticulum (Cho *et al.*, 2011; White *et al.*, 2017b). Both cell organelles are pivotal in host membrane trafficking and known to be hijacked by intracellular bacteria (Asrat *et al.*, 2014).

The final stage of horizontal transmission involves successful invasion of the new host population. This depends on maternal transmission efficiency, strength of reproductive parasitism, and direct fitness effects on the host. When introduced into a new host, however, *Wolbachia* frequently exhibit low transmission efficiency (Clancy and Hoffmann, 1997; Heath *et al.*, 1999; Rigaud *et al.*, 2001; Riegler *et al.*, 2004). Moreover, there are cases in which reproductive parasitism is too weak to ensure successful invasion, not to mention the CI-associated minimum infection frequency below which invasion is not possible. In such cases, an initially rare infection should not be able to invade. How, then, could *Wolbachia* spread so successfully and infect thousands and thousands of host species? A possible answer lies in positive fitness effects temporarily bestowed upon the host to come through the dire straits of population invasion (after successful invasion, reproductive parasitism should, in theory, be sufficient to maintain the infection).

1.2.6 Mutualistic effects

Although there is no need for *Wolbachia* to become mutualistic (due to their reproductive parasitism), it still pays for them to evolve traits that increase host fitness, as long as those traits do not hinder their own transmission. A mutant strain that, in addition to manipulating host reproduction, confers some fitness benefit to the host is at an advantage over non-mutualistic strains (Turelli, 1994). Hence, even reproductive parasites are in principle selected to enhance host fitness. Indeed, recent years have seen a growing

body of evidence suggesting that *Wolbachia* can increase the fitness of their arthropod hosts, for example through nutritional provisioning or anti-pathogenic protection. In particular, anti-pathogenic effects of *Wolbachia* have been arousing great interest, primarily because of the potential to use these effects to control mosquito-borne disease (Iturbe-Ormaetxe *et al.*, 2011; Caragata *et al.*, 2016). In addition to those facultative benefits, hosts can become dependent on their symbionts (obligate mutualism). A textbook example of such evolved dependence is the wasp *Asobara tabida* that requires *Wolbachia* for oogenesis (Dedeine *et al.*, 2001). In Chapter 4, we present a comprehensive review of *Wolbachia* mutualisms in arthropod hosts, including both facultative and obligate relationships. We focus on a critical assessment of anti-pathogenic effects and their biological relevance and on the diverse relationships between mutualisms and reproductive manipulations. Based on the clear evidence of *Wolbachia*-associated direct fitness benefits, we present, in Chapter 5, a mathematical model of the effects of such benefits on *Wolbachia* infection dynamics, thus unifying and extending earlier theoretical studies (e.g., Fenton *et al.* 2011; Kriesner *et al.* 2013). Our findings corroborate that mutualistic behavior considerably contributes to *Wolbachia*'s great evolutionary success.

1.3 Scope of this thesis

The present thesis is structured as follows. In Chapter 2, we present an estimate of how many arthropod species are infected with *Wolbachia*. To this end, we use a beta-binomial model developed by Hilgenböcker *et al.* (2008) and apply it to a more appropriate data set. Our analysis reveals that a major part of all terrestrial arthropod species is infected, suggesting that *Wolbachia* thrive in millions of species. We discuss in detail why we think our estimate is more reasonable than earlier ones. Lastly, we also estimate infection frequencies of several other reproductive parasites and compare them to the estimated incidence of *Wolbachia*.

In order to gain a better understanding of this great pandemic, in Chapter 3, we combine epidemiological theory with network theory to analyze *Wolbachia* interspecies transmission dynamics over evolutionary time. This requires a conceptual modification: in our model, we consider species, not individuals, as infectious agents. *Wolbachia* infection hence spreads on a contact network of arthropod host species. More specifically, we choose a small-world network which idealizes the known relationships between *Wolbachia* transmission and host phylogeny. Our model is able to explain

Wolbachia's high incidence levels commonly found in nature, and it also makes several predictions on the evolutionary dynamics of *Wolbachia* infection frequency among arthropod species. Finally, we point to open questions concerning *Wolbachia* interspecies transmission.

In our epidemiological model, horizontal transmission between species brings about high incidence rates for *Wolbachia* even without explicitly assuming beneficial effects of infection. However, infection of novel host species requires successful population invasion, and beneficial effects are probably crucial to achieve that. In Chapter 4, therefore, we comprehensively review the evidence of mutualistic effects of *Wolbachia* on their arthropod hosts. This includes both facultative mutualisms (where the host does not depend on the symbiont, but benefits from its presence) and obligate ones (where the symbiont is required for host reproduction or survival). In a thorough analysis of *Wolbachia*'s anti-pathogenic effects, we find that many of them are more likely a byproduct than a directly selected trait, and that there is so far only limited evidence of *Wolbachia*-induced protection in the field. These reservations notwithstanding, we find a plethora of cases in which *Wolbachia* indeed positively affect host fitness.

In Chapter 5, we substantiate the impact of beneficial effects on the evolution of *Wolbachia* by means of a population genetic model. In particular, we are interested in the effects of direct fitness benefits on the infection dynamics of reproductive parasites, using the examples of cytoplasmic incompatibility and male-killing. We derive invasion conditions and equilibrium infection frequencies for different invasion scenarios. In line with previous theory, we find that direct fitness benefits clearly facilitate invasion, and hence a critical step in the infection of novel hosts. Our results therefore suggest that direct fitness benefits substantially contribute to the evolutionary success of reproductive parasites.

Endosymbiosis and host immunity are highly interrelated. However, many immunological aspects of *Wolbachia*–arthropod interactions are only poorly understood. For example, it is unclear how the arthropod immune system is involved in the diverse phenotypes of *Wolbachia*, and why host resistance is not observed more frequently. In Chapter 6, we give an overview of the interactions between *Wolbachia* and the host immune system, with a special focus on reactive oxygen species. We propose a hypothesis concerning the effects of *Wolbachia* on the immune system of novel and coevolved hosts. This hypothesis offers a mechanistic explanation of several *Wolbachia*-induced phenotypes, including anti-pathogenic effects.

2 How many species are infected with *Wolbachia*?—An update informed by better data

Recently, a statistical analysis estimated the infection frequency of *Wolbachia* among arthropods to be 66%. At the same time, the authors of this analysis highlighted some weaknesses of the underlying data and concluded that in order to improve the estimate, a larger number of individuals per species should be assayed and species be chosen more randomly. In this chapter, we apply the statistical approach to more appropriate data from a survey that tested both a broad range of species and a sufficient number of individuals per species. Indeed, we find a substantially different infection frequency: we now estimate the proportion of *Wolbachia*-infected species to be around 40% which is lower than the previous estimate but still points to a surprisingly high number of arthropods harboring the bacteria. Notwithstanding this difference, we confirm the previous result that, within a given species, typically most or only a few individuals are infected. We discuss extensively why several common procedures of *Wolbachia* screenings are likely to yield biased estimates of the infection frequency. Since the survey analyzed here largely avoids these drawbacks, our analysis probably provides a more reasonable estimate of *Wolbachia* incidence. Moreover, we extend our analysis to include several other reproductive parasites and corroborate that *Wolbachia* are the most abundant endosymbionts among arthropod species.

Most parts of this chapter have been published in *PLoS ONE* (Zug and Hammerstein, 2012).

2.1 Introduction

Both the proportion of infected individuals within species (prevalence) and the overall percentage of infected species (incidence) are important parameters describing the infection frequency of *Wolbachia*. In order to estimate these parameters, HILGENBÖCKER *et al.* (2008) recently presented a meta-analysis that combined the data from 20 *Wolbachia* screenings with more than 900 arthropod species in total. Using a statistical approach, i.e. a beta-binomial model, they found that prevalences are typically very low or very high, and estimated the incidence of *Wolbachia* to be around 66%, which is considerably higher than previous estimates of approximately 20% (WERREN *et al.*, 1995a; WERREN and WINDSOR, 2000). A major reason for such underestimation is the sampling of only one or a few individuals per species. With these one-individual samples, low (and even high) prevalence infections are likely to be overlooked. On the other hand, HILGENBÖCKER *et al.* (2008) found that samples comprising more than 100 individuals per species tend to be biased towards infected species, e.g. due to prior knowledge of infection. Although they corrected for the latter bias by excluding particularly large samples, many studies used in their meta-analysis still included quite a lot of one-individual samples and were restricted to specific host taxa (see HILGENBÖCKER *et al.*, 2008 for details). Therefore, in order to more accurately assess the incidence of *Wolbachia* in arthropod hosts, it is crucial to analyze a data set that comprises a medium number of individuals from randomly chosen species. Here, we apply the approach by HILGENBÖCKER *et al.* (2008) to data from a recent survey by DURON *et al.* (2008) that meets these requirements more closely. This survey also tested for the presence of several other reproductive parasites, which allows us to estimate incidences of other endosymbionts and compare them to that of *Wolbachia*.

2.2 Model

In the survey by DURON *et al.* (2008), 136 species of terrestrial arthropods (2052 individuals in total) were screened for the presence of seven reproductive parasites: *Wolbachia*, *Arsenophonus*, *Cardinium*, *Flavobacterium*, *Rickettsia*, *Spiroplasma ixodetis* and *S. poulsonii*. Since *Flavobacterium* was never observed, we excluded it from our analysis. In the survey, not more than 40 individuals were sampled per species, and in only 25 of the 136 species tested, less than 10 individuals were sampled (median: 15 individuals

per species; mode: 20 individuals per species). This range of sampled individuals should help to avoid the drawbacks of both one-individual samples and the bias associated with extensive sampling. Arthropod species tested encompassed 15 orders and three classes (Insecta, Arachnida, Malacostraca), thus representing a widespread and sufficiently random collection. Taken together, the data from DURON *et al.* (2008) should satisfy the requirements for an improved data set as outlined above.

We again use the framework of a beta-binomial model to estimate symbiont prevalence q and incidence x. Different species are assumed to exhibit different prevalences, and thus q values follow a probability distribution $p(q)$. The incidence x is then estimated by integrating the prevalence distribution:

$$x = \int_c^1 p(q)\ dq, \tag{2.1}$$

where c defines a threshold frequency below which species are considered to be uninfected. For a more detailed account of the model, see HILGENBÖCKER *et al.* (2008).

2.3 Results and discussion

The prevalence distribution for *Wolbachia* shows that either most or only few individuals within a species are infected (Figure 2.1). Based on this distribution, *Wolbachia* incidence is estimated to be $x = 0.406$ for $c = 0.001$ (Table 2.1). We chose $c = 0.001$ in accordance with HILGENBÖCKER *et al.* (2008) to facilitate comparisons. Our results confirm the main qualitative findings from the previous meta-analysis, i.e. the 'most-or-few' prevalence pattern and the likely underestimation of incidence in previous *Wolbachia* screenings. However, there is one major difference between the results of the two analyses: In the first study, *Wolbachia* incidence was estimated to be around 66% (for $c = 0.001$). Based on the data from DURON *et al.* (2008), we now obtain a lower estimate of the percentage of *Wolbachia*-infected species, i.e. approximately 40%. We think that our current estimate is more reasonable for the following three reasons.

First, the underlying data contain only a very low proportion of species samples in which only a few individuals were tested. Testing only a small number of individuals considerably increases the likelihood of randomly picking some uninfected individuals from an actually infected species, particularly if prevalence levels are low. Indeed, there is evidence that infection frequencies within species are often variable between geographically distinct

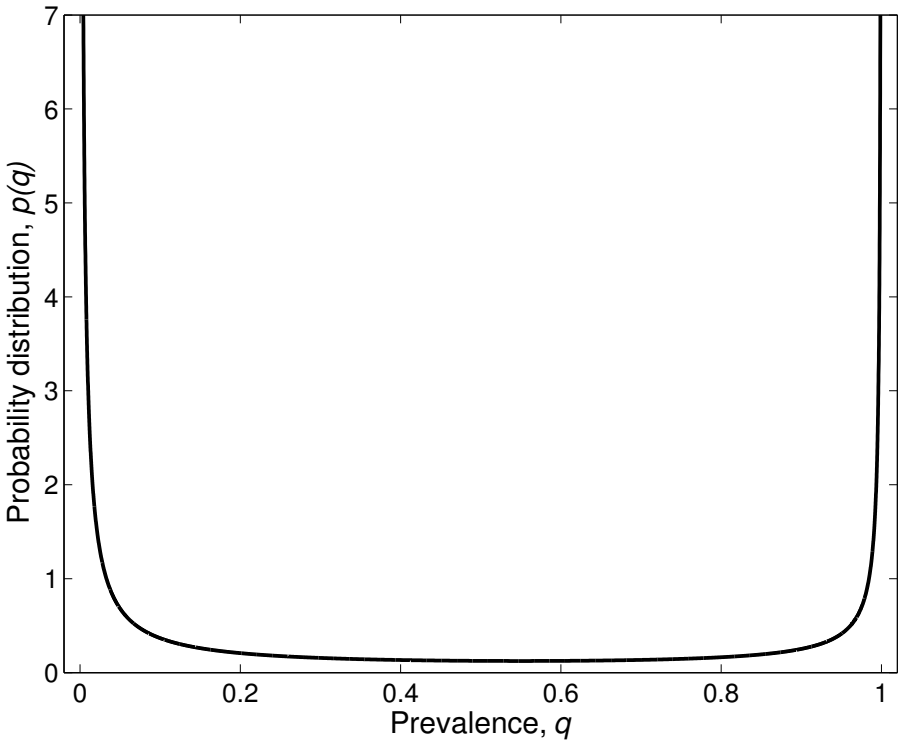


Figure 2.1: Estimated probability distribution of *Wolbachia* prevalence.

populations. Such a prevalence variation between populations was found in several species-specific surveys, ranging from 0% to 100% in the cherry fruit fly or from 4% to 100% in two planthoppers (Arthofer *et al.*, 2009b; Hughes *et al.*, 2011a). In another fruit fly screening that tested 1500 individuals, only extremely low prevalence levels were found among different populations, ranging from 0% to 3% (Sun *et al.*, 2007). Moreover, species might also be falsely classified as uninfected because of low-titer infections that are not detected. Recent evidence suggests that such low-titer *Wolbachia* infections within arthropod hosts are more common than previously thought (Arthofer *et al.*, 2009a; Hughes *et al.*, 2011a). Taken together, sampling more than just a few individuals, as it was predominantly done by Duron *et al.* (2008), avoids the pitfalls outlined above and thus significantly improves estimates of *Wolbachia* infection frequencies in nature.

A second reason why we think our current estimate is more accurate is that the new data set does not include large samples (not more than

Table 2.1: Estimates of incidence x of different endosymbionts, depending on threshold infection frequency c.

	Incidence x		
	$c = 0.01$	$c = 0.001$	$c = 0.0001$
Wolbachia	0.335	0.406	0.470
Arsenophonus	0.059	0.066	0.072
Cardinium	0.111	0.162	0.211
Rickettsia	0.014	0.061	0.114
Spiroplasma ixodetis	0.148	0.221	0.289
S. poulsonii	0.022	0.032	0.041

40 individuals per species). Large samples are likely to be biased towards infection, probably because respective species were already known to be infected and were sampled extensively to study infection prevalence in more detail. Additionally, large samples will disproportionately often be samples of common species, just because common species are more easily collected in large amounts (Hilgenböcker *et al.* 2008; cf. the collecting procedure in Weinert *et al.* 2007). Common species, however, are again prone to have already been tested for infection. These are important issues because large samples inherently have a strong impact on the estimation procedure. Therefore, as was already pointed out by Hilgenböcker *et al.* (2008), omission of large samples will improve estimates of *Wolbachia* incidence.

Thirdly, the fact that Duron *et al.* (2008) sampled a wide range of arthropod species from 15 different orders should render this collection sufficiently diverse in phylogenetic terms. In contrast to the previous meta-analysis which pooled the results from many taxon-specific screenings, analyzing a broad taxon survey ensures that all species are examined with the same detection method. Usually, *Wolbachia* infections are detected by PCR assays which crucially depend on the sensitivity of commonly used PCR primers. A recent assessment of standard PCR protocols used for *Wolbachia* detection, however, reveals considerable variation in primer efficiency (Simões *et al.*, 2011). To summarize, the data set compiled by Duron *et al.* (2008) is the first one that satisfies our criteria for a reliable estimate (no one-individual samples, no large samples, no restriction to a specific host taxon). In contrast, several *Wolbachia* screenings that have since been published fail to satisfy the criteria and are not included in our analysis

(see, for example, Wiwatanaratanabutr *et al.*, 2009; Kondo *et al.*, 2011; Yun *et al.*, 2011; Doudoumis *et al.*, 2012; Evison *et al.*, 2012). We therefore think that our estimate of *Wolbachia* incidence within arthropods is more reliable than previous attempts. Another reason for the difference in incidence estimates between our study and that by Hilgenböcker *et al.* (2008) might be the different sampling range. As described above, infection frequencies within species can differ greatly between geographically distinct populations. Duron *et al.* (2008) pointed out that such geographical variation in prevalence was likely to increase when expanding the sampling range beyond Western Europe, where species were predominantly collected. In contrast, the meta-analysis by Hilgenböcker *et al.* (2008) comprises samples from most continents, covering both temperate and tropical zones. Although speculative, this unequal geographical sampling might partially explain the difference in incidence estimates derived from both data sets.

Previous broad taxon surveys of *Wolbachia* infection frequencies among arthropods found approximately 20% of the tested species to be infected (Werren *et al.*, 1995a; Werren and Windsor, 2000). In general, previous surveys have estimated incidence by dividing the number of infected species by the overall number of species tested. Adopting the same straightforward approach to the data by Duron *et al.* (2008) yields a very similar estimate (22.8%). However, this is roughly only half of our 40% estimate, although based on the same data set. Therefore, the proportion of *Wolbachia*-infected species seems to be considerably higher than a first glance would suggest. In order to compare the infection frequency of *Wolbachia* to that of other reproductive parasites, we estimate the incidence for five endosymbionts that were also included in the survey by Duron *et al.* (2008). Since these symbionts were detected only in very few species, the graphic representation of the prevalence distributions is of limited value and therefore not displayed here. Incidence levels range from 0.032 (*Spiroplasma poulsonii*) to 0.221 (*Spiroplasma ixodetis*; Table 2.1, all values for $c = 0.001$). Again, our estimates are consistently higher than those obtained by the straightforward approach (see Duron *et al.*, 2008). Yet, even *S. ixodetis* as the most common of these other symbionts does not match *Wolbachia* in terms of incidence, which corroborates the status of *Wolbachia* as the most abundant reproductive parasite of arthropod hosts. Considering the species richness of the global arthropod community, with an estimated 6.8 million species of terrestrial arthropods (Stork *et al.*, 2015), our estimate implies that millions of species are infected with *Wolbachia*. Thus, although lower than estimated by Hilgenböcker *et al.* (2008), the number of species harboring *Wolbachia* is still remarkably high, making them "one of the great pandemics

in the history of life" (Werren *et al.*, 2008).

The beta-binomial approach developed by Hilgenböcker *et al.* (2008) has since been applied repeatedly to estimate *Wolbachia* incidence (Ahmed *et al.*, 2013; Weinert *et al.*, 2015; Sazama *et al.*, 2017). The database of the most comprehensive meta-analysis to date contains over 150 000 individuals from over 3500 arthropod species (Weinert *et al.*, 2015). This analysis estimates that 52% of all arthropod species are infected with *Wolbachia*, which is fairly similar to our estimate. However, the data set underlying this meta-analysis exhibits several drawbacks mentioned in this chapter, such as many one-individual samples, many large samples, and different primer sensitivities. The authors were well aware of that and put a lot of effort into correcting for diverse sampling biases. Nevertheless, it is not clear how comparable their findings are to ours. Since the two other meta-analyses (Ahmed *et al.*, 2013; Sazama *et al.*, 2017) contain similar weaknesses, we believe that the data underlying our estimate are the most balanced ones to date.

3 Horizontal transmission of *Wolbachia* between arthropod host species

The abundance of *Wolbachia* among arthropods is all the more impressive considering that infections are suggested to be frequently lost within host species due to the evolution of resistance. This apparent paradox suggests that horizontal transmission between host species has been a key factor in shaping the *Wolbachia* pandemic. Since *Wolbachia* infections are thus acquired and lost like any other infection, in this chapter, we use a standard epidemiological model to analyze *Wolbachia* horizontal transmission dynamics over evolutionary time. Conceptually modifying the model, we apply it not to transmission between individuals but between species. Since, in evolutionary time, infections spread frequently between closely related species and occasionally over large phylogenetic distances, we represent the set of host species as a small-world network which satisfies both requirements. We find that the ratio between transmission rate and recovery rate is crucial for determining the proportion of infected species (incidence). Our results also point to the importance of occasional transmission over long phylogenetic distances for the observed high incidence levels of *Wolbachia*. In conclusion, we are able to explain why *Wolbachia* are so abundant among arthropods although selection for resistance within hosts often leads to infection loss.

A slightly different version of this chapter has been published in the *Journal of Evolutionary Biology* (Zug *et al.*, 2012).

3.1 Introduction

Despite the evolutionary success of *Wolbachia*, there is broad evidence for loss of infection in host lineages over evolutionary time, probably due to selection for resistance. Suppressor alleles have been found in hosts infected by feminizing or male-killing *Wolbachia* (Rigaud and Juchault, 1992; Hornett *et al.*, 2006). In the case of CI, theory predicts that male-specific suppressor genes reducing the level of CI spread within populations, probably leading to the eventual loss of *Wolbachia* infection (Koehncke *et al.*, 2009). Accordingly, *Wolbachia* infection rapidly decays with increasing age in *Aedes albopictus* males, but not in females (Tortosa *et al.*, 2010), and further evidence in line with the evolution of CI suppressor genes in males comes from *Nasonia longicornis* (Raychoudhury and Werren, 2012). Moreover, CI intensity is a neutral trait and may thus degenerate over time, leading to eventual loss of *Wolbachia* even without evolution of host resistance (Hurst and McVean, 1996). Lastly, CI strains are highly susceptible to invasion and replacement by strains that induce parthenogenesis, feminization or male-killing, which in turn could be lost from the population (Hurst *et al.*, 2002). As a result, most reproductive manipulations seem to imply the eventual extinction of the manipulators, rendering *Wolbachia* infections evolutionarily transient within a given host lineage.

These findings present a paradox: although bound for extinction within arthropod host species, *Wolbachia* have become extremely abundant among them. This suggests that, in addition to vertical transmission, *Wolbachia* are also transmitted horizontally between species over evolutionary time. The idea that horizontal transmission can solve this paradox is not new (Hurst *et al.*, 1992; Hurst and McVean, 1996),[1] and a simple model of *Wolbachia* horizontal transmission was presented by Werren and Windsor (2000). As we have seen in the Introduction, evidence of horizontal transmission comes from a lack of congruence between *Wolbachia* and host phylogenies, and several different mechanisms have been proposed to account for such interspecies transmission events. Since most of these mechanisms do not require close relatedness between donor and recipient species and since incongruence in phylogenies often involves large phylogenetic distances, these findings strongly suggest that *Wolbachia* are able to move between distantly related host species.

[1] As Hurst and McVean (1996) have noted, this idea rests on a clade selection argument: although there is no short-term selection for increased horizontal transmission rates, clade selection favors those *Wolbachia* lineages that have the ability to undergo horizontal transmission; those lineages will persist over evolutionary time.

Still, the probability of successful horizontal transmission of *Wolbachia* likely is highest between closely related hosts and decreases with increasing phylogenetic distance. *Wolbachia*–host specialization was found in different arthropod taxa (Jiggins *et al.*, 2002a; Russell *et al.*, 2009; Schuler *et al.*, 2013). Evidence from interspecific transfer experiments also identifies close phylogenetic relatedness between hosts as important for successful horizontal transmission (Heath *et al.*, 1999; Van Meer and Stouthamer, 1999; Huigens *et al.*, 2004; Riegler *et al.*, 2004). Similarly, hybrid introgression—another means by which *Wolbachia* may cross the species boundary (Jiggins, 2003; Raychoudhury *et al.*, 2009)—is presumably restricted to closely related species (Mallet, 2005). Combining these findings on interspecies transmission of *Wolbachia* suggests that transmission occurs predominantly between closely related species, for example within genera, and occasionally between distantly related hosts, for example even between members of different insect orders.

Altogether, both acquisition and loss are hallmarks of *Wolbachia* infection dynamics on evolutionary timescales (Frost *et al.*, 2010). For a particular host species, infection patterns are thus cyclical over evolutionary time (Koehncke *et al.*, 2009). First, *Wolbachia* would infect a species through horizontal transmission and spread through the new host by vertical transmission from generation to generation. Second, selection for resistance in hosts would at some point lead to loss of the infection. Third, the now costly but redundant suppressor would be selected against or degenerate through mutation, thus rendering the species susceptible to reinfection. Because maintenance of the *Wolbachia* pandemic depends on the rate of horizontal transmission between species and on the rate of infection loss within species, epidemiology provides a convenient means of analyzing *Wolbachia* infection dynamics (Werren and Windsor, 2000; Werren *et al.*, 2008). Furthermore, "tracing the network of *Wolbachia* movements" (Werren *et al.*, 2008) requires characterizing the contact network structure of arthropod hosts that underlies these movements.

In this chapter, we combine standard epidemiological theory with network theory to investigate *Wolbachia* transmission dynamics between arthropod hosts. We choose a simple stochastic compartment model (i.e. the SIRS model) that captures the cyclical character of infections within species over evolutionary time as well as the random character of infection gains (horizontal transmission, hybrid introgression) and losses (evolution of resistance). Applying this model to the *Wolbachia* pandemic requires a conceptual adaptation because we regard species, rather than individuals, as infectious agents. Accordingly, the host assemblage in question is not a population,

but a set of species. Because transmission occurs predominantly between phylogenetically neighbouring host species but also occasionally between distantly related species, we represent the assemblage of arthropod hosts as a small-world network that incorporates both properties: neighboring nodes represent closely related species, with each node being connected to several neighbors, whereas distant nodes represent distantly related species that are interconnected only with low probability.

We stress that this small-world network is neither a phylogenetic network nor a representation of a phylogenetic tree or a cladogram. Instead, it is a transmission network that accounts for the described relationships between *Wolbachia* transmission and host phylogeny. As such, a given network does not even need to represent a complete phylogenetic entity (genus, family, etc), but might comprise only a subset or an assemblage of species of different taxa (e.g. insects in a tropical rice-field community; KITTAYAPONG *et al.*, 2003). In any case, species can be arranged as a string of phylogenetically nearest neighbours and thus be represented by the open-ring lattice of the small-world network.

We show that horizontal transmission of *Wolbachia* between arthropod species can be adequately adressed by using epidemiology in evolutionary time. First, this unorthodox approach readily explains the high proportion of infected species, that is, the high incidence of *Wolbachia* among arthropods. Depending on host network size and time available for the infection to spread, our findings further suggest that, in many cases, incidence levels may still be increasing on evolutionary timescales. Finally, we show that the absence of long-range connections within the host network can lead to a sharp decrease in incidence levels, implying that occasional transmission events over long phylogenetic distances have been crucial in both shaping and maintaining the global *Wolbachia* pandemic.

3.2 Model

3.2.1 Small-world network

The two key features of *Wolbachia* interspecies movements are clustered transmission among phylogenetically neighboring species (i.e. many short-range movements) and rare transmission over long phylogenetic distances (i.e. few long-range movements). Due to insufficient data, it is not possible to trace the actual host contact network that underlies these movements. We therefore use an idealized, computer-generated network that satisfies the criteria of having many short-range and few long-range transmission

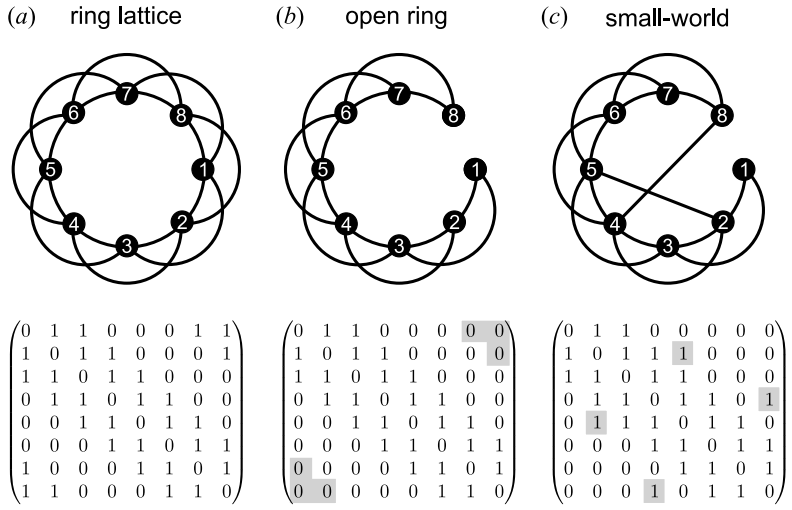


Figure 3.1: The three steps of generating the open small-world network. For each step, a network scheme and the underlying adjacency matrix are shown. The number of nodes is $N = 8$ in this schematic. Matrix elements that have changed compared to the previous matrix are shaded in grey. See text for details of the generating process. (a) A regular ring lattice with four edges per node. (b) The open-ring lattice. (c) The open small-world network with, in this example, two random long-range connections.

occasions, that is, a small-world network. A network is said to have small-world properties if it is highly clustered (if node i is connected to node j and node j is connected to node l, then, with high frequency, i is connected to l) and has short average path lengths (it takes only a few steps to move along the shortest path between two randomly selected nodes; Watts and Strogatz, 1998). This small-world network represents the host species assemblage, with nodes denoting host species and edges representing connections between species on which *Wolbachia* infection can pass via horizontal transmission or introgressive hybridization. The high clustering property translates into having many short-range connections, and the short path length property corresponds to having few long-range connections.

We depict the small-world network as an open ring lattice (i.e. a ring lattice with a break) with random long-range connections (Figure 3.1c). *Wolbachia* transmission events between distantly related host species are assumed to be represented by long-range connections; those between neighboring species by short-range connections, that is, connections between a node and its nearest

neighbors along the ring lattice. Thus, we assume the topological distance between two nodes on the ring lattice to correspond to the phylogenetic distance between the respective species, with the break representing the location at which the most distantly related species meet topologically. We ignore host speciation (including *Wolbachia*–host co-divergence, which is a rare event; Raychoudhury *et al.*, 2009) and extinction, as well as species migration, which results in a static species network with constant connections over time. We represent a network of N nodes by a binary $N \times N$ adjacency matrix $\boldsymbol{A}$, with $a_{i,j} = 1$ if there is an edge between nodes i and j, and $a_{i,j} = 0$ otherwise. We assume the network to be undirected (transmission along an edge is possible in both directions) and simple (there are no loops connecting a species directly to itself and no more than one edge between any two different nodes). Based on these assumptions, $\boldsymbol{A}$ is a symmetric matrix with zero diagonal: $a_{i,j} = a_{j,i}$, and $a_{i,i} = 0$.

We generate a small-world network in three steps. First, we create a regular ring lattice with N nodes and k edges per node, with $\boldsymbol{A}$ being represented by a symmetric Toeplitz matrix (Figure 3.1a). Second, in order to insert the break, we set the non-zero entries in the lower left and upper right corner of the Toeplitz matrix equal to zero (Figure 3.1b). Third, we add random cross-connections to the open-ring lattice by adding, with probability p, non-zeros to $\boldsymbol{A}$ at random locations, but preserving its symmetry and excluding non-zero diagonal entries to prevent loops (Figure 3.1c). Probability p corresponds to the rate at which long-range connections are added so that, on average, the total number of added long-range cross-connections is Np. This procedure, adapted from Higham and Higham (2005), is slightly different from the rewiring method used in the original work by Watts and Strogatz (1998), but has the advantage of preserving all regular short-range edges along the ring lattice, which is more adequate for our purposes. In constructing the small-world network, we always chose $k = 4$ for the number of nearest neighbors (which is a conservative estimate because many species are likely to have more than four closely related species to whom *Wolbachia* might be transmitted) and $p = 0.1$ for the probability of adding random connections (which is also a conservative estimate within the small-world regime from $p = 0$ [regular graph] to $p = 1$ [random graph]).

3.2.2 SIRS model

To simulate the spread of *Wolbachia* infection on a small-world host network, we use the SIRS (Susceptible–Infected–Recovered–Susceptible) model,

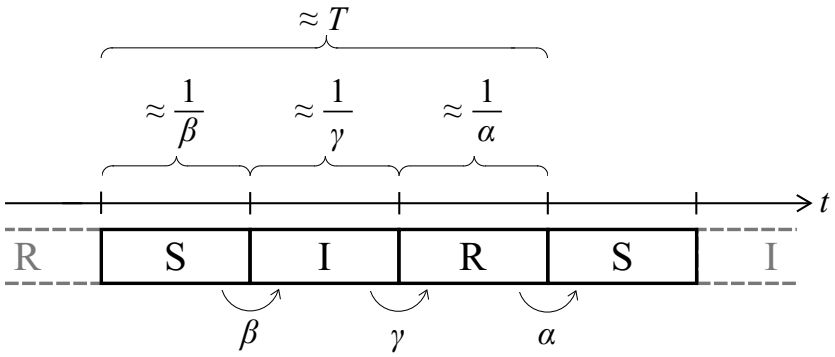


Figure 3.2: Schematic illustration of the SIRS model. In evolutionary time, a host species passes successively through the **S** stage, the **I** stage, the **R** stage, again the **S** stage and so on. The corresponding transition rates β, γ and α are indicated. Note that the actual rate for the transition from **S** to **I** is '$\beta \times$ number of infectious contacts', but may be approximated by β. The reciprocals of the transition rates determine the average time periods spent in the respective stage. The sum of all three average time periods is denoted by T.

which is a standard epidemiological compartment model classifying hosts by their disease status (Keeling and Rohani, 2008). Species in the **S** compartment are susceptible to *Wolbachia* infection and can become infected with transmission rate β through an infected species with whom they have contact. Species in the **I** compartment have been infected and can spread the infection (via horizontal transmission or hybrid introgression). Due to selection for resistance, species lose the infection with recovery rate γ, move to the **R** compartment and pass a period of temporary immunity (resistance). Eventually, species lose their resistance to *Wolbachia* with immunity loss rate α, thus becoming susceptible again and returning to the **S** compartment (see Figure 3.2 and Table 3.1 for possible transitions and their corresponding rates). The SIRS model is especially well suited to represent the cyclical pattern of *Wolbachia* infection dynamics within host clades over evolutionary time. The possibility of multiple infections, as well as mutualistic relationships between *Wolbachia* and their arthropod hosts, which might pose barriers to horizontal transmission and infection loss, will not be considered here.

Considering a set of host species of size N, the number of species in each compartment at time t is given by $S(t)$, $I(t)$, and $R(t)$, respectively: $S(t) + I(t) + R(t) = N$. We assume that the time period spent in each compartment is exponentially distributed so that the average periods can

Table 3.1: Possible events, transitions and rates in the SIRS model.

Event	Transitions	Rate
Infection	$S(t) \to S(t) - 1$, $I(t) \to I(t) + 1$	Transmission rate β $\times$ number of infectious contacts
Loss of infection	$I(t) \to I(t) - 1$, $R(t) \to R(t) + 1$	Recovery rate γ
Loss of resistance	$S(t) \to S(t) + 1$, $R(t) \to R(t) - 1$	Immunity loss rate α

be approximated by the reciprocals of the epidemiological parameters: the average infectious period is given by $1/\gamma$, and the average period of temporary immunity by $1/\alpha$. The average period of susceptibility may be approximated by the reciprocal of the product of β and the number of infectious contacts. Because the number of overall contacts per given node is relatively small (as we used $k = 4$), the number of infectious contacts is even smaller. As β values are extremely small, multiplication with the number of infectious contacts will not significantly change the magnitude of the β values. Therefore, we can use $1/\beta$ as a rough approximation of the average period of susceptibility (Figure 3.2). This implies that the average overall time necessary to pass through a single infection cycle (T) is approximately equal to the sum of all three reciprocals: $T \approx \frac{1}{\beta} + \frac{1}{\gamma} + \frac{1}{\alpha}$.

3.2.3 Stochastic simulations

Although the SIRS model can be described deterministically by a system of differential equations, there are two main reasons to choose a stochastic network model for our purposes. First, both interspecies horizontal transmission events and evolution of resistance to *Wolbachia* are rare and random events. Second, as outlined above, the spatial structure of the small-world network better accounts for *Wolbachia* transmission routes between species than does the homogenous-mixing assumption of the deterministic model which states that each individual has equal chance of transmitting the infection to any other individual. As a result, we use a stochastic version of the SIRS model to simulate the spread of a *Wolbachia* infection across a small-world network over time. A convenient method for tackling this issue is provided by the Gillespie algorithm, which is a procedure for generating time-evolution trajectories of finite populations in continuous time

(Gillespie, 1977). Here, we follow the approach by Keeling and Rohani (2008).

We consider a network (species assemblage) of size N. Every node in the network is represented by its current infection state. The state vector $\mathbf{s}(t) = (s_1(t), \ldots, s_N(t))$ assigns to every node $i = 1, \ldots, N$ its infection state at time t, with

$$s_i(t) = \begin{cases} 1 & \text{if node } i \text{ is susceptible at time } t \\ 2 & \text{if node } i \text{ is infectious at time } t \\ 0 & \text{if node } i \text{ is recovered at time } t. \end{cases} \tag{3.1}$$

Given an initial time t_0 and assuming that node $i = 1$ is the only infectious one, the initial state is given by $s_1(t_0) = 2$ and $s_l(t_0) = 1$, where $l = 2, \ldots, N$. The transition probability of every node i at time t is captured by the rate vector $\mathbf{r}(t) = (r_1(t), \ldots, r_N(t))$, with

$$r_i(t) = \begin{cases} \beta \sum_{j=1}^{n_i} H_{c_j^i} & \text{if } s_i(t) = 1 \\ \gamma & \text{if } s_i(t) = 2 \\ \alpha & \text{if } s_i(t) = 0. \end{cases} \tag{3.2}$$

Again, β is the transmission rate, γ the recovery rate, and α the immunity loss rate. H_j is equal to one if node j is infectious; otherwise, H_j is zero. $c_1^i, c_2^i, \ldots, c_{n_i}^i$ are the n_i contacts of node i. Therefore, $\sum_{j=1}^{n_i} H_{c_j^i}$ is the number of infectious contacts by which node i can become infected. Considering the initial state of $\mathbf{r}(t)$, we have one single infectious node which is node $i = 1$; therefore, $r_1(t_0) = \gamma$. All other nodes are susceptible. Every susceptible node that is connected to node 1 is assigned $r(t_0) = \beta$, all other susceptible nodes have $r(t_0) = 0$.

With $\mathbf{s}(t_0)$ and $\mathbf{r}(t_0)$ at hand, we start the update of $\mathbf{s}(t)$ to see how the fractions of susceptible, infectious, and recovered nodes change over time. According to the Gillespie algorithm, we determine (i) when the next transition occurs, and (ii) which node m is the next one performing this transition and thus changing its state $s_m(t)$. Let p_1, p_2 be two random numbers drawn from the uniform distribution in the unit interval, and $r_0 = \sum_i r_i(t)$. The time step to the next transition τ is an exponentially distributed random number scaled by the sum of all transition rates, r_0:

$$\tau = \frac{1}{r_0} \ln(\frac{1}{p_1}). \tag{3.3}$$

The index of the next node that changes its state is

$$m = \text{the smallest integer satisfying} \sum_{i=1}^{m} r_i(t) > p_2 r_0. \tag{3.4}$$

This procedure ensures that the next node at which a transition occurs is drawn randomly, but proportional to $r_i(t)$. The time is advanced by τ, and $\mathbf{s}(t)$ is updated according to

$$s_m(t+\tau) = \begin{cases} 1 & \text{if } s_m(t) = 0 \\ 2 & \text{if } s_m(t) = 1 \\ 0 & \text{if } s_m(t) = 2. \end{cases} \tag{3.5}$$

As for the update of $\mathbf{r}(t)$, we have to distinguish three cases:

1. The changing node m has just lost its immunity and is again susceptible: $s_m(t+\tau) = 1$. Therefore,

$$\begin{aligned} r_m(t+\tau) &= \beta \times \text{number of infectious contacts} \\ &= \beta \sum_{j=1}^{n_i} H_{c_j^i}. \end{aligned} \tag{3.6}$$

2. The changing node m has just become infected: $s_m(t+\tau) = 2$. Therefore,

$$r_m(t+\tau) = \gamma \tag{3.7}$$
$$r_v(t+\tau) = r_v(t) + \beta, \tag{3.8}$$

   where the index v represents all susceptible nodes that are connected to the node m.

3. The changing node m has just recovered: $s_m(t+\tau) = 0$. Therefore,

$$r_m(t+\tau) = \alpha \tag{3.9}$$
$$r_v(t+\tau) = r_v(t) - \beta. \tag{3.10}$$

After the update of both $\mathbf{s}(t)$ and $\mathbf{r}(t)$, the process is iterated as long as $I(t) > 0$ and $t < t_{max}$.

Every simulation consisted of generating a network (open small-world or open ring) and iterating the Gillespie algorithm. Due to the network's random connections, repeating network creation in every simulation increases

the robustness of results because it lowers the influence of statistically outlying small-world network topologies (i.e. extreme numbers of random connections). Infections started from a single infectious node chosen randomly from all N nodes with all other $N-1$ nodes initially susceptible. The Gillespie algorithm was iterated as long as $I(t) > 0$ and $t < t_{max}$ (t_{max} being the maximum time value for a simulation run), and every simulation was run 500 times (except for Figures 3.5 and 3.6 where simulations were run 100 times [10 times for three values in Figure 3.5] to save computational time).

Our main interest was in estimating the proportion of infected species I^* (i.e. the incidence) which we calculated as follows: at the end of each simulation run, we divided the final number of infected species by the number of species in the network, thereby obtaining I_s^* (s for a single simulation run): $I_s^* = I(t_{max})/N$. By taking into account only those simulations that ran to t_{max}, we excluded those in which infection disappeared globally. After the simulation had been run 500 (100) times, we averaged I_s^* over all simulation runs, thus giving I^* (in case the infection disappeared in all simulation runs, we set $I^* = 0$). The incidence I^* reached its endemic equilibrium when I^* did not increase significantly with increasing t_{max} values.

Programs were written, and simulations were performed using Matlab 7.6 (The MathWorks, Inc.) and C++ (with Visual Studio 2010, Microsoft), with some code partly based on Keeling and Rohani (2008). All data were analyzed using Matlab.

3.3 Results

3.3.1 Estimation of epidemiological parameters

How frequently *Wolbachia* move between species in evolutionary time is difficult to estimate. There are, however, estimates of the age of infection or the most recent species-wide sweep of *Wolbachia* in different host species (varying between 3000 and 700 000 years; Table 3.2). This time frame provides a reasonable benchmark for the average duration of the infectious period ($1/\gamma$) and thus for the recovery rate γ, for which we choose values varying between 10^{-6} and 10^{-3} per year. We assume the transmission rate β to be of a similar order of magnitude as γ. The reasons for this assumption are that (i) infections will quickly disappear if $\beta \ll \gamma$ and (ii) successful horizontal transmissions between two randomly chosen arthropod species are rare events so that $\beta \gg \gamma$ seems equally unlikely. Furthermore, we assume the rate of immunity loss α to be higher than β and γ because a

Table 3.2: Estimated age of *Wolbachia* infection or of the most recent sweep in different host species.

Host species	Estimated age (years)	Reference
Hypolimnas bolina	< 3000	Duplouy *et al.* (2010)
Acraea encedana	< 16 000	Jiggins (2003)
Culex pipiens	12 000–21 000	Atyame *et al.* (2011)
Chelymorpha alternans	100 000–125 000	Keller *et al.* (2004)
Drosophila innubila	15 000–700 000	Jaenike and Dyer (2008)

costly but superfluous resistance allele will decline quickly due to mutation or selection against it. For t_{max}, we chose different values with an upper limit of 10 million years, which should be a sufficiently large time frame to make reasonable predictions within the overall evolutionary history of *Wolbachia* among arthropods, whose origin has recently been estimated to be about 200 million years ago (Gerth and Bleidorn, 2016).

3.3.2 Influence of the epidemiological parameters

We first consider the influence of the transmission rate β, recovery rate γ, and immunity loss rate α on the incidence of *Wolbachia* I^*, always varying only the rate in question, leaving all other parameters unchanged. As expected, increasing transmission of *Wolbachia* between species (β) positively affects incidence I^* (Figure 3.3a), whereas more frequent infection loss within species (γ) negatively affects I^* (Figure 3.3b). In the model context, this is because β is the only parameter which directly replenishes the compartment of infectious species (**I**), whereas γ is the only parameter to directly deplete it (Figure 3.2).

In the deterministic version of the SIRS model, the basic reproduction number (a key parameter in epidemiology) is given by $R_0 = \beta/\gamma$, and major epidemic outbreaks are possible only if $R_0 > 1$. Our network model reproduces this threshold property of the β/γ ratio. Due to the spatial structure of the small-world network, positive incidence levels are possible even if $\beta/\gamma < 1$, although infections are more likely to occur if $\beta > \gamma$, as measured by the breadth of the respective β/γ parameter range. Moreover, we can use the ratio β/γ as an indicator of how both parameters influence the incidence I^*. As expected, I^* is an increasing function of the ratio β/γ (Figure 3.3d).

The immunity loss rate α has a similar positive effect on the incidence I^*

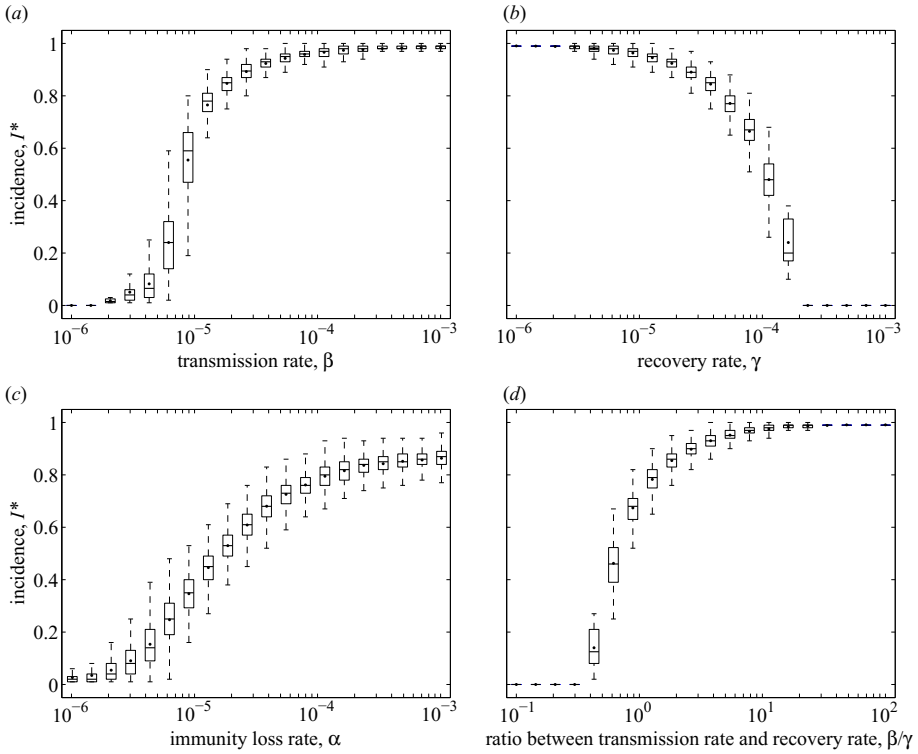


Figure 3.3: The influence of (*a*) transmission rate β, (*b*) recovery rate γ, (*c*) immunity loss rate α, and (*d*) the ratio between transmission rate and recovery rate β/γ on incidence I^*. Shown are representative results for each case. Each I^* value was calculated independently with 500 simulation runs per value. Bold dots and lines indicate means and medians, respectively; boxes show lower and upper quartiles; error bars encompass data within 1.5 times the interquartile range. $N = 100$ in all panels; $t_{max} = 10^6$ years (*a*–*c*), 5×10^6 years (*d*); rate values: (*a*) $\gamma = 10^{-5}$, $\alpha = 10^{-3}$, (*b*) $\beta = 8 \times 10^{-5}$, $\alpha = 10^{-3}$, (*c*) $\beta = 2 \times 10^{-5}$, $\gamma = 10^{-5}$, (*d*) $\beta = 10^{-5}$, γ values ranging from 10^{-4} to 10^{-7} (from left to right), $\alpha = 10^{-3}$. All rates are given per year.

as the transmission rate β, though with a more gradual increase (Figure 3.3c). This is because the effect of α on I^* is indirect as it first replenishes the **S** compartment which then refills the **I** compartment (Figure 3.2). Moreover, when a critical range for α is exceeded, increasing α even further will not change I^* anymore, even if I^* has not reached 100% (Figure 3.3c). To investigate this further, we chose three different β/γ ratios (10, 1, and 0.5), increased α up to 0.1 and also increased t_{max} fivefold to rule out possible time limitations. Even under these altered conditions, and for each β/γ ratio tested, the incidence does not change with increasing α (data not shown). This finding is especially relevant because we estimated α to often be significantly higher than β and γ (see above). In this case ($\alpha \gg \beta, \gamma$), the fact that varying α has no substantial effect on I^* makes a more accurate estimation of α unnecessary and thus increases the robustness of our results with respect to α. Overall, these results suggest that the ratio between transmission rate and recovery rate is crucial in determining the proportion of *Wolbachia*-infected species.

To investigate this ratio-dependent effect further, we take up the three β/γ ratios (10, 1, and 0.5), but additionally include α to get three constant ratios between all three rates: β:γ:α = 10:1:100, 1:1:100, and 1:2:100. We measure the absolute magnitude of the parameters β, γ and α using the average duration of a single infection cycle T ($T \approx \frac{1}{\beta} + \frac{1}{\gamma} + \frac{1}{\alpha}$). If we keep a constant ratio between the three parameters, I^* does not change significantly, regardless of the absolute magnitude of β, γ and α (data not shown). Therefore, it is possible to obtain every particular incidence level by a large number of parameter combinations, which partially compensates for the lack of data regarding parameter estimation.

3.3.3 Temporal infection dynamics and influence of host assemblage size

In order to investigate the effects of the evolutionary time available t_{max} on the incidence I^*, we use the three previous β/γ ratios of β/γ = 10, 1, and 0.5. With increasing time available, I^* increases for all three ratios; the higher the β/γ ratio, the faster the increase (Figure 3.4). Within the first 10^4 years, I^* values are similarly low for all three ratios, and roughly between 10^4 and 10^6 years, I^* values increase. Beyond a certain t_{max} value, the incidence has reached its endemic equilibrium and does not increase anymore. These results suggest that *Wolbachia* infection must have been spreading for tens of thousands of years or longer to achieve the equilibrium state. In host networks existing for less than millions of years, *Wolbachia*

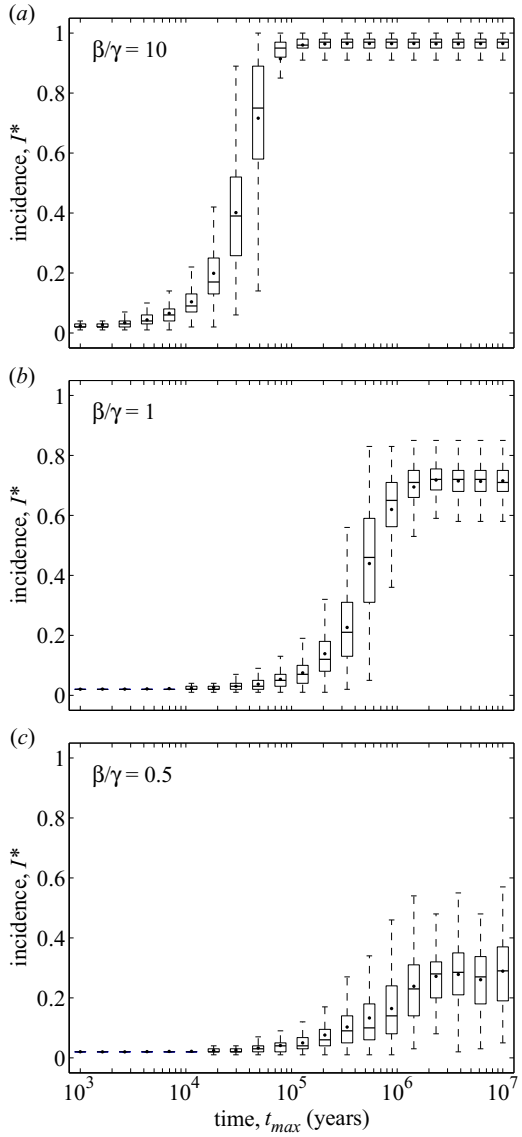


Figure 3.4: The influence of available time t_{max} on incidence I^*. Shown are results for three β/γ values: (a) $\beta/\gamma = 10$, (b) $\beta/\gamma = 1$, and (c) $\beta/\gamma = 0.5$. Each I^* value was calculated independently with 500 simulation runs per value. Bold dots and lines indicate means and medians, respectively; boxes show lower and upper quartiles; error bars encompass data within 1.5 times the interquartile range. $N = 100$ in all panels; (a) $\beta = 10^{-4}$, $\gamma = 10^{-5}$, $\alpha = 10^{-3}$, (b) $\beta = \gamma = 10^{-5}$, $\alpha = 10^{-3}$, and (c) $\beta = 10^{-5}$, $\gamma = 2 \times 10^{-5}$, $\alpha = 10^{-3}$. All rates are given per year.

symbionts might thus still be in a pre-equilibrium state, implying bacterial incidence to still be increasing on an evolutionary timescale.

Assessing the influence of network size N on the temporal dynamics of the incidence I^* demonstrates that, if time available t_{max} is sufficient so that I^* can reach equilibrium, increasing N has almost no effect on I^*. With less time available, however, I^* decreases with increasing N (Figure 3.5). We conclude that in evolutionarily ancient *Wolbachia* infections (i.e. t_{max} large), even networks consisting of millions of host species are very likely to not significantly differ in incidence levels from those we investigated here, that is, networks with up to 10^5 species. On the other hand, the effect that in evolutionarily young infections (t_{max} small) *Wolbachia* tend to accomplish only low incidence levels is considerably enhanced in larger host assemblages.

3.3.4 Removing the long-range connections

Occasional transmission events between distantly related host species may be one reason for the global distribution of *Wolbachia* among arthropod hosts and for their evolutionary success in general. We use our model to test the possible role of long-range connections as an amplifier of *Wolbachia* incidence. To this end, we let infection spread on an open-ring lattice lacking long-range connections (Figure 3.1b) and compared the results to those from the small-world network. Within the first 10^5 years of infection, there is no significant difference between the small-world and open-ring network: for both network structures, the incidence is hardly above 0%. Subsequently, however, the increase in I^* is considerably slower on the open-ring lattice than on the small-world network (Figure 3.6a). This negative impact of the missing long-range links becomes even stronger with decreasing β/γ ratio (Figure 3.6b) and increasing network size (data not shown). We conclude that, across a wide parameter space and particularly within large host networks (including networks larger than those studied here), long-range connections are a prerequisite for *Wolbachia* to achieve the high incidence levels commonly found in nature.

3.4 Discussion

We applied an epidemiological network model to *Wolbachia* horizontal transmission between arthropod species over evolutionary time. The study was motivated by the contradictory findings that a large percentage of all arthropod species is estimated to be infected with *Wolbachia* (see Chapter 2) but

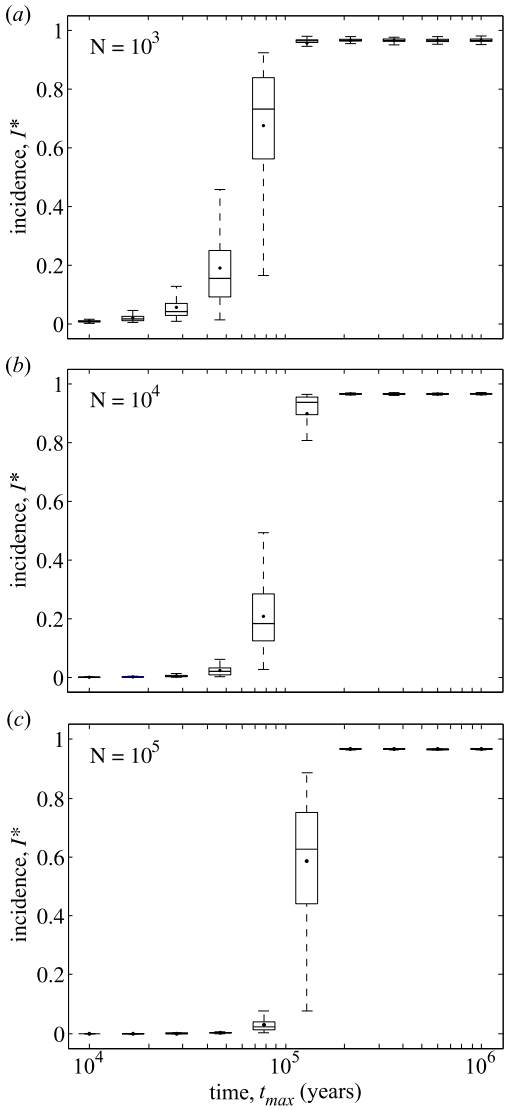


Figure 3.5: The influence of network size N and available time t_{max} on incidence I^*. Shown are results for a network of size (a) $N = 10^3$, (b) $N = 10^4$, and (c) $N = 10^5$. Each I^* value was calculated independently with 100 simulation runs per value (except the last three values in (c) which were calculated with 10 runs per value). Bold dots and lines indicate means and medians, respectively; boxes show lower and upper quartiles; error bars encompass data within 1.5 times the interquartile range. $\beta = 10^{-4}$, $\gamma = 10^{-5}$, $\alpha = 10^{-3}$ (and thus $\beta/\gamma = 10$). All rates are given per year.

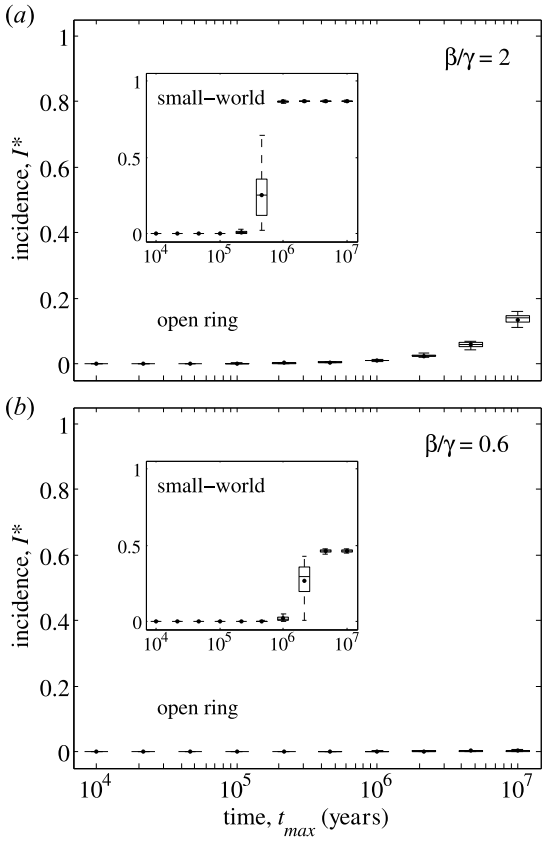


Figure 3.6: The influence of long-range connections on incidence I^*. Shown are results as a function of available time t_{max} for open-ring (without long-range connections) and small-world network (with long-range connections; inset) for (*a*) $\beta/\gamma = 2$ and (*b*) $\beta/\gamma = 0.6$. Each I^* value was calculated independently with 100 simulation runs per value. Bold dots and lines indicate means and medians, respectively; boxes show lower and upper quartiles; error bars encompass data within 1.5 times the interquartile range. $N = 10^4$ in both panels; (*a*) $\beta = 2 \times 10^{-5}$, $\gamma = 10^{-5}$, $\alpha = 10^{-3}$; (*b*) $\beta = 10^{-5}$, $\gamma = 1.67 \times 10^{-5}$, $\alpha = 10^{-3}$. All rates are given per year.

Table 3.3: Model predictions on the evolutionary dynamics of *Wolbachia* incidence.

The ratio between the rates at which species acquire and lose *Wolbachia* is the main determinant of *Wolbachia* incidence. The rate at which species lose resistance to *Wolbachia* is of minor importance
In host communities with an evolutionarily young *Wolbachia* infection, incidence is likely to still be increasing on an evolutionary timescale. This effect is enhanced in larger host communities
In host communities with an evolutionarily ancient infection, incidence is likely to be at equilibrium. The size of host communities has little effect
Wolbachia transmission over large phylogenetic distances will often be crucial for obtaining high incidence levels. Hybrid introgression, which works only over small phylogenetic distances, will often be insufficient to obtain such high incidence

that infections are likely to be lost on evolutionary timescales (Koehncke *et al.*, 2009). This apparent paradox can be resolved if one considers cyclical infection patterns within host clades over time. This is, to our knowledge, the first attempt to use epidemiological modeling to investigate *Wolbachia* interspecies infection dynamics, and our results suggest that these infection dynamics can be adequately addressed by such modeling. By computer simulations, we show that (i) the ratio between transmission rate β and recovery rate γ is the major determinant of *Wolbachia* incidence levels in host species assemblages, (ii) the evolutionary time available for *Wolbachia* to spread may considerably limit their incidence, especially in large host assemblages, and (iii) occasional transmission events over long phylogenetic distances are, under a wide range of conditions, indispensable for *Wolbachia* to achieve similar incidence levels as observed in nature (see Table 3.3 for a summary of predictions).

Our first main finding—the pivotal role of β/γ in determining the incidence of *Wolbachia*—reflects the importance of one of the key quantities in epidemiology, the basic reproduction number R_0 (most basically defined as β/γ). R_0 can be used as a threshold quantity that determines whether an epidemic occurs (Keeling and Rohani, 2008). We are able to reproduce the threshold behavior of the incidence as a function of β/γ. A recent co-phylogenetic analysis involving more than 1,000 arthropod species estimates

the *Wolbachia* acquisition rate β to be 0.11 per million years, and the loss rate γ to be 0.14 per million years (Bailly-Bechet *et al.*, 2017). These estimates are approximately one order of magnitude smaller than our lower bound for these rates (1 per million years). What is crucial, however, is the ratio between these rates; their β/γ ratio is 0.79 and thus very similar to ratios that yield reasonable results in our model (e.g. $\beta/\gamma = 1$ in Figure 3.4b or $\beta/\gamma = 0.6$ in Figure 3.6b). Our finding that the effect of immunity loss rate α on incidence levels is negligible, at least in the estimated parameter range, confirms the significance of transmission and recovery rate. Moreover, this result confers considerable robustness to our approach because (i) we need not further confine the parameter range for α and (ii) many different parameter combinations yield the same incidence levels. As a result, the limited knowledge of actual parameter values does not preclude an epidemiological approach to *Wolbachia* infections, particularly because basic findings from standard epidemiology (e.g. the role of β/γ) also apply to the *Wolbachia* pandemic.

Second, our model predicts that a particular incidence level encountered in nature may still be in a pre-equilibrium state, and that incidence levels should increase with evolutionary time before finally reaching equilibrium. The question of whether *Wolbachia* incidence within global arthropod communities is at equilibrium or increasing was already put forward by Werren and Windsor (2000) and again addressed by Bailly-Bechet *et al.* (2017). Our results support the latter possibility and thus suggest that host species assemblages with an evolutionarily ancient *Wolbachia* infection should tend to have a higher incidence than host assemblages that have acquired the infection only recently. The limiting effect of time becomes particularly relevant in large host assemblages where *Wolbachia* takes longer to reach equilibrium incidence levels. However, one should not conclude that incidence levels generally tend to decrease with increasing host assemblage size. On the contrary, a recent meta-analysis of species-level phylogenies found a positive correlation between clade age and species richness (McPeek and Brown, 2007). Thus, larger host clades are likely to have an older age compared to smaller clades and thus also have a higher chance to have acquired *Wolbachia* at an earlier stage. This could partially compensate for the incidence-decreasing effect of increasing clade size.

A third key result of our study is the finding that *Wolbachia* movement between distantly related host species is often necessary to reach naturally occurring incidence levels, particularly in large host assemblages. Besides different modes of horizontal transmission, introgressive hybridization has been suggested as a means for *Wolbachia* to move between species (Jiggins,

Table 3.4: Open questions concerning parameters of *Wolbachia* interspecies transmission.

Parameter	Comments
Transmission rate β	**How frequently does a species acquire *Wolbachia* infection in evolutionary time?** Can this rate be extrapolated from data on population-wide sweeps in ecological time? To what extent will this rate be species-specific (e.g. depend on physiological and/or ecological background)?
Recovery rate γ	**How frequently does a species lose *Wolbachia* infection in evolutionary time?** To what extend will this rate depend on the type of reproductive manipulation induced by *Wolbachia*?
Immunity loss rate α	**How fast does the functionality of suppressor alleles decline within a species in the absence of *Wolbachia*?** Can this rate be inferred from population-genetic analyses of the fate of redundant genes?

2003; Raychoudhury *et al.*, 2009). However, only a fraction of hybridization events is accompanied by introgression of *Wolbachia*. Moreover, the vast majority of hybrid introgression events are presumably restricted to transmission within genera because hybridization beyond the genus level appears to be rare in insects (Mallet, 2005). Thus, if we consider a network that consists of several genera, hybrid introgression alone (corresponding to the open-ring lattice that lacks any long-range connections) is not sufficient to obtain adequate levels of *Wolbachia* incidence. Our findings therefore point to the importance of transmission mechanisms other than hybrid introgression in order to explain the empirically observed incidence levels.

The idea of regarding species as individual infectious agents on an evolutionary timescale and within an epidemiological framework is essential to our model. At the same time, its high level of abstraction makes it difficult to validate our model with empirical data because both the 'species-as-infectious-agents' conception and the consequent lack of data impede parameter estimation. Some open questions regarding parameter estimation are listed in Table 3.4. However, as pointed out previously, our incidence estimates often depend more on parameter ratios than on absolute values, which partially compensates for difficulties in parameter estimation and

thus allows the high abstraction level.

Our model assumes that the three epidemiological parameters are equal for all host species within a network. Regarding the transmission rate, this assumption overlooks that the physiological and ecological background of different arthropod species might promote or impede horizontal transmission. For example, generalist parasitoids and predators probably have a higher chance of transmitting and/or acquiring *Wolbachia* than specialists (Rokas *et al.*, 2002). Regarding the recovery rate, the particular reproductive phenotype that is induced by *Wolbachia* influences the probability of being lost from the host. Whereas resistance to *Wolbachia* is likely to eventually evolve in the case of CI, male-killing and feminization, host species that depend on thelytoky-inducing *Wolbachia* for reproduction (see Chapter 4) do not seem to easily lose their symbionts. One might speculate, however, that allowing for species-specific differences in transmission rate and recovery rate will probably primarily increase the variance of incidence and have less effect on the incidence level itself. Therefore, ignoring these differences should be a reasonable simplification.

In our model, we consider neither host speciation nor extinction. Including these processes, however, is not likely to change our findings qualitatively. This is because both speciation and extinction affect network size N, whose influence on *Wolbachia* incidence we already investigated. Moreover, regardless of whether a species is in the susceptible (**S**), infectious (**I**) or recovered (**R**) compartment, both speciation and extinction can be understood as a replenishment or depletion of the particular compartment and, therefore, as a change in the transition rate (β, γ, α) replenishing or depleting that compartment (Fig 3.2). For example, the emergence of uninfected species can be understood as a replenishment of the **S** compartment, which in turn can be expressed as an increase in α. The extinction of infected species can be expressed as an increase in γ and so on. Because we analysed all effects of changing transition rates, we believe that our model delivers insightful results without the explicit inclusion of speciation or extinction events.

In a similar effort though with a different focus, Engelstädter and Hurst (2006b) investigated how incidence dynamics of horizontally transmitted parasites depend on the topology of host phylogenies. Assuming that transmission probability declines exponentially with increasing phylogenetic distance, they show that heterogeneity in phylogenetic history (i.e. symmetric vs. asymmetric trees) can lead to heterogenous incidence between clades. Our approach differs from theirs in several ways. First, by including spatial structure using a small-world transmission network, we overcome the assumption that transmission probability solely depends

on phylogenetic distance, thereby specifically including transmission over large distances as is strongly suggested by phylogenetic evidence. Second, by explicitly adopting a standard epidemiological model, we incorporate more suitably the cyclical pattern of *Wolbachia* infections within species and introduce a productive method for future research in this direction.

Our study reveals that the application of epidemiology in evolutionary time advances our understanding of the *Wolbachia* pandemic among the global arthropod community. We show that horizontal transmission between unrelated hosts, including cases of large phylogenetic distance, is necessary to account for the high incidence found in many host clades, but also that incidence levels are likely to further increase on evolutionary timescales. These insights would have not been possible if one considers *Wolbachia* as purely vertically transmitted parasites. When conceptualizing species as infectious agents, epidemiological modeling thus provides a productive new perspective to analyze *Wolbachia*–host systems and their evolutionary infection dynamics. Our approach can be applied not only to *Wolbachia*, but to all parasites that move both vertically within and horizontally between host species, such as other bacterial endosymbionts (Jaenike *et al.*, 2007; Chiel *et al.*, 2009; Weinert *et al.*, 2009; Duron *et al.*, 2010; Ros *et al.*, 2012) and even viruses (Longdon *et al.*, 2011). Future studies could profit from epidemiology, particularly combined with network theory, to further elucidate the evolutionary transmission dynamics between endosymbionts and their hosts.

4 A critical assessment of *Wolbachia* mutualisms in arthropod hosts

There has been a recent upsurge in reports on *Wolbachia*-associated fitness benefits. Therefore, the question arises how such instances of mutualism are related to the phenotypes of reproductive parasitism. In this chapter, we review the evidence of *Wolbachia* mutualisms in arthropods, including both facultative and obligate relationships, and critically assess their biological relevance. Although many studies report anti-pathogenic effects of *Wolbachia*, only few of them actually prove these effects to be relevant to field conditions. We further show that *Wolbachia* frequently have beneficial and detrimental effects at the same time, and that reproductive manipulations and obligate mutualisms may share common mechanisms. These findings undermine the idea of a clear-cut distinction between *Wolbachia* mutualism and parasitism. In general, both facultative and obligate mutualisms can have a strong, and sometimes unforeseen, impact on the ecology and evolution of *Wolbachia* and their arthropod hosts. Acknowledging this mutualistic potential might be the key to a better understanding of some unresolved issues in the study of *Wolbachia*–host interactions.

A slightly different version of this chapter has been published in *Biological Reviews* (Zug and Hammerstein, 2015a).

4.1 Introduction

Even for reproductive parasites it can pay to enhance host fitness. In theory, any trait that increases the fitness of infected females will increase transmission through these females and hence will be selected. Indeed, recent years have witnessed rapid accumulation of evidence suggesting that *Wolbachia* can have positive effects on the fitness of their arthropod hosts and thus behave as mutualists, both of the facultative and obligate type (Figure 4.1; see Table 4.1 for definitions). Mutualistic relationships between *Wolbachia* and arthropods can be as intimate as the ancient mutalisms between *Buchnera* and aphids or *Wigglesworthia* and tsetse flies: in the bedbug *Cimex lectularius*, for example, *Wolbachia* reside in a specialized host organe, the bacteriome, and provide essential nutrients (Hosokawa *et al.*, 2010; Figure 4.1A). The fact that *Wolbachia*-induced fitness benefits can occur in the presence or absence of a reproductive manipulation prompts the question of how both effects are related to each other. In other words, are *Wolbachia* in arthropod hosts parasitic, mutualistic, or both? Moreover, considering potential benefits of *Wolbachia* infection might be helpful in elucidating several other outstanding issues. For example, how can *Wolbachia* persist in novel host species, although they initially often perform poorly in new hosts? Why has host resistance to *Wolbachia* been found only so rarely, given that selection would act on hosts to suppress reproductive parasites? And can *Wolbachia* become ultimate mutualists (see Table 4.1), so that the host performs better than it would ever have done without the bacteria?

In this chapter, we gather evidence of *Wolbachia* mutualisms in arthropods and thus outline possible answers to these questions. We first describe phenotypes of facultative mutualism and conditions that are favorable for its emergence, with a special emphasis on *Wolbachia*-mediated protection against pathogens. Next, we provide evidence of obligate mutualism induced by *Wolbachia* in arthropod hosts and discuss how different forms of dependence may have evolved. To this end, we present three case studies on the evolution of dependence in order to highlight common features as well as differences between them. Finally, we sketch possible evolutionary fates of *Wolbachia*–arthropod mutualisms and outline directions for future research.

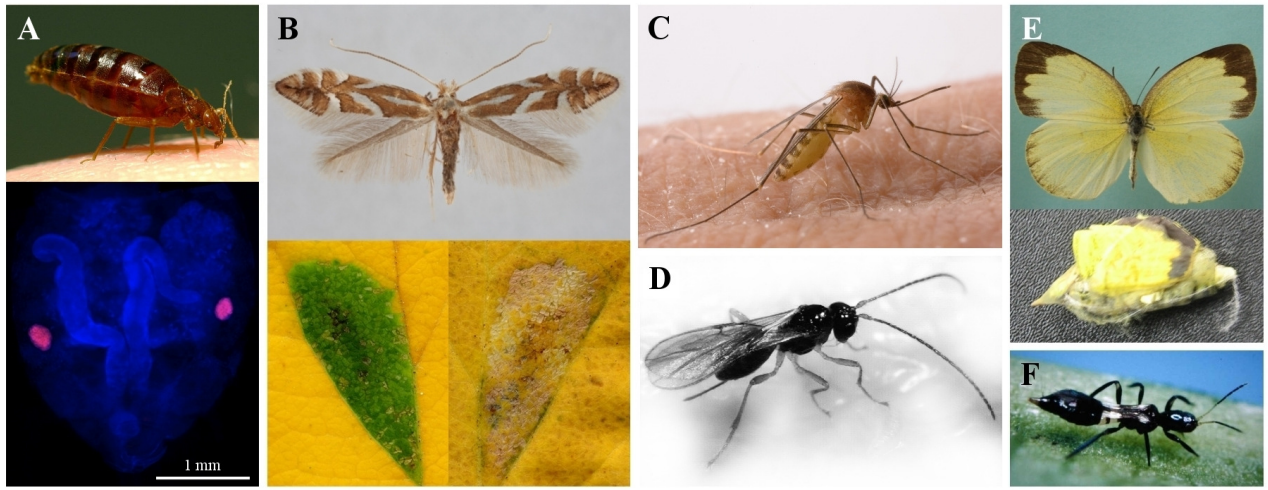


Figure 4.1: *Wolbachia* mutualisms in arthropod hosts. (A) In the bedbug *Cimex lectularius* (top), *Wolbachia* provide essential B vitamins and are housed in specialized organs, the bacteriomes (bottom, magenta spots). © Dr. Richard Naylor, cimexstore.co.uk (top), Takahiro Hosokawa (bottom). (B) The leaf miner *Phyllonorycter blancardella* (top) relies on *Wolbachia* to cope with nutritional constraints in senescent leaves. Infected larvae are able to induce so-called 'green-islands' (bottom left), whereas cured larvae are not (bottom right). © Bert Gustafsson (top), David Giron (bottom). (C) The mosquito *Culex pipiens* is naturally infected with *Wolbachia* and the pathogen *Plasmodium relictum*. *Wolbachia* protects its host against *Plasmodium*-induced mortality. © Hans M. Smid, bugsinthepicture.com. (D) The parasitic wasp *Asobara tabida* depends on *Wolbachia* for oogenesis. © Kees Hofker. (E) The butterfly *Eurema mandarina* (top) is infected with feminizing *Wolbachia*. After larval antibiotic treatment, many adults show an intersexual phenotype, fail to escape from the pupal case and die (bottom). It is possible, though, that intersexual defects, rather than the lack of *Wolbachia*, are the cause of death. © Daisuke Kageyama. (F) In *Franklinothrips vespiformis*, *Wolbachia*-induced parthenogenesis has led to the complete loss of sexual function, making the symbiont an obligate mutualist for daughter production. © Entocare, Wageningen NL.

Table 4.1: Definitions of mutualism-related terms used in this review.

Term	Definition
Mutualism	A symbiotic relationship in which both partners (host and symbiont) benefit
Parasitism	A symbiotic relationship in which one partner benefits at the expense of the other
Facultative mutualism[a]	A mutualistic relationship in which the symbiont is not necessary for successful host development or reproduction, but if it is present, the host enjoys some benefit from it
Obligate mutualism[a]	A mutualistic relationship in which the symbiont is required for host reproduction or survival
Proximate mutualism	A mutualistic relationship in which symbiont removal results in a decreased performance of the host. Proximate mutualisms can be the result of either ultimate mutualism or evolved dependence
Ultimate mutualism[b]	A mutualistic relationship in which the host could never have performed as well without the symbiont, i.e. the host gains some 'real' benefit from the interaction. In practice, detecting ultimate mutualisms is difficult because two different host genotypes must be compared: an infected host that is adapted to the presence of its symbiont must perform better than an uninfected conspecific that is adapted to the symbiont's absence
Evolved dependence[b]	A mutualistic relationship in which the host has lost the ability to perform well in the absence of its symbiont. Evolution of dependence is a precursor to obligate mutualism
'Jekyll and Hyde' infection	A symbiotic relationship in which a reproductive parasite simultaneously acts as a mutualist
'Stand-alone benefit' infection	A symbiotic relationship in which symbiont-associated benefits occur without any reproductive manipulation

[a] Both in facultative and obligate mutualisms, the endosymbiont benefits because it cannot survive outside of the host cell.

[b] For a more detailed discussion on ultimate mutualism and evolved dependence, see De Mazancourt *et al.* (2005).

4.2 From parasitism to mutualism

Originally, the idea of fitness-enhancing *Wolbachia* was launched by recurrent findings showing that the infection can be prevalent within a population even though reproductive manipulation is low or absent (Giordano *et al.*, 1995; Hoffmann *et al.*, 1996, 1998; Perrot-Minnot *et al.*, 2002; Charlat *et al.*, 2003; Bouwma and Shoemaker, 2011). Theory suggests that, in such cases, *Wolbachia* should increase host fitness in order to be maintained. Turelli (1994) showed that selection on CI-inducing *Wolbachia* favours variants that increase the relative fecundity of infected females, even if these variants reduce the strength of CI. Under different conditions, however, selection on fecundity-enhancing strains is likely to preserve CI. Thus, once selection for increasing fecundity is operating, *Wolbachia* might either continue to manipulate host reproduction (case I), or not (case II). In case I, *Wolbachia* simultaneously act as a beneficial symbiont and as a reproductive parasite—a situation called 'Jekyll and Hyde' infection (Jiggins and Hurst, 2011; see Table 4.1). It has been shown that beneficial effects of CI-inducing *Wolbachia* facilitate their invasion and spread in host populations (Dobson *et al.*, 2002; Fenton *et al.*, 2011), making 'Jekyll and Hyde' infections good candidates for particularly successful *Wolbachia* strains. Moreover, such infections blur the distinction between mutualistic and parasitic *Wolbachia* (Herre *et al.*, 1999; Sachs *et al.*, 2011a).

In case II, *Wolbachia*-associated benefits occur without reproductive manipulations. These 'stand-alone benefit' infections are likely to exhibit larger net benefits than 'Jekyll and Hyde' infections and are perhaps the best candidates for ultimate mutualisms (see Table 4.1), although it is difficult to prove that a given relationship actually reflects an ultimate mutualism (De Mazancourt *et al.*, 2005). Although speculative, the ability to induce a reproductive phenotype might only be hidden behind the beneficial trait and might suddenly become visible, for example after a host shift. Such a hiding effect has not yet been demonstrated for beneficial *Wolbachia* traits, but it has been shown that the ability to induce one reproductive manipulation can be hidden by another (Hornett *et al.*, 2008). Therefore, it is possible that 'stand-alone benefit' infections might easily turn into 'Jekyll and Hyde' infections.

By showing that, under certain circumstances, reproductive parasites are selected to become increasingly benign, the analysis by Turelli (1994) provides theoretical evidence for the notion that mutualistic *Wolbachia* evolved from parasitic ancestors (transition 1 in Figure 4.2). This view is supported by more general studies on the origins of bacterial mutualism (Ewald, 1987;

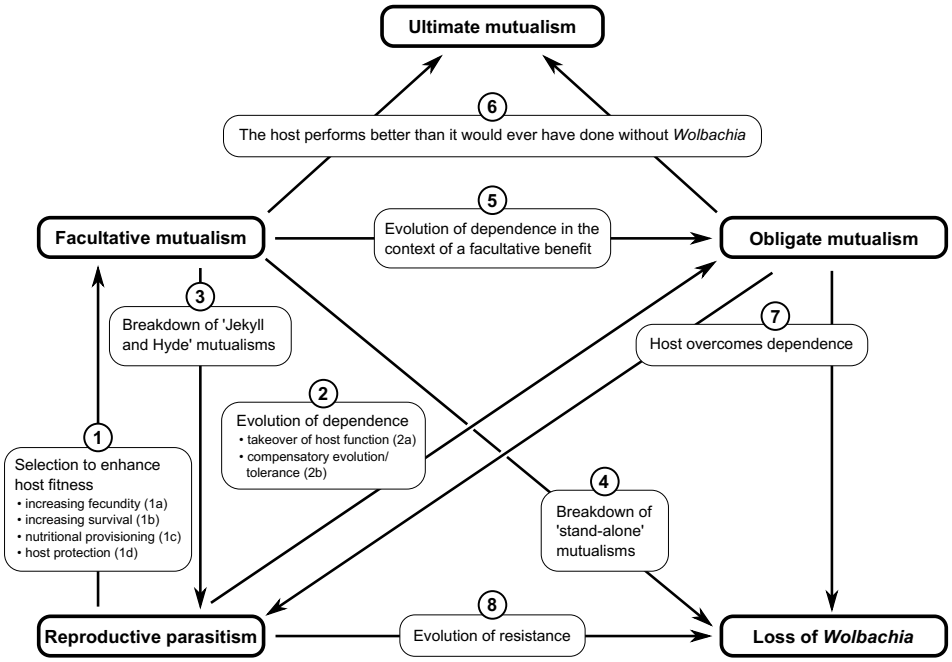


Figure 4.2: A schematic overview of the possible transitions between different symbiotic relationships of *Wolbachia* and arthropod hosts. Each transition is depicted by an arrow and explained by the overlying numbered box (box 6 and box 7 belong to two transitions each). The numbers correspond with the descriptions of the transitions in the text. 'Jekyll and Hyde' mutualisms are those which occur together with a reproductive manipulation, whereas 'stand-alone' mutualisms do not. Note that the overview is non-exhaustive and also makes no statements on how likely each transition is. See text for further details.

Sachs *et al.*, 2011b). Accordingly, transitions from parasitism to mutualism have been found in several *Wolbachia*–arthropod associations (Vavre *et al.*, 1999b; Fry *et al.*, 2004; Weeks *et al.*, 2007). Among the several phenotypes of reproductive parasitism, CI is probably the best candidate for a hypothetical starting point for a transition from parasitism to mutualism. In contrast to the sex-ratio distorting phenotypes, CI causes selection on females to improve bacterial transmission because *Wolbachia*-free females suffer from incompatibility with infected males (Koehncke *et al.*, 2009). This selection for high vertical transmission is likely to have two effects: Firstly, it favours fixation of CI-inducing *Wolbachia* within populations; once near fixation, CI does little damage because most individuals are infected and thus protected

from the phenotype. Secondly, high vertical transmission enables the host to adapt to the presence of *Wolbachia*. The fact that both effects promote the evolution of mutualism makes CI the most likely parasitic ancestor of a mutualistic phenotype (Engelstädter and Hurst, 2009).

4.3 *Wolbachia* as facultative mutualists

4.3.1 Overview

In this section, we provide an overview of beneficial *Wolbachia* phenotypes that are facultative from the host's point of view, that is, although hosts benefit from infection, they do not depend on *Wolbachia* for survival or fecundity. Therefore, infected individuals can be cured of infection by antibiotic treatment or introgression crosses (but see Section 4.5 for some shortcomings of antibiotic treatment as a method to identify *Wolbachia* effects). A straightforward way to examine *Wolbachia*-induced fitness effects is to compare survival or fecundity rates of infected versus uninfected females. Due to maternal inheritance of *Wolbachia*, there is no selection to increase male fitness (although there are a few cases known in which *Wolbachia* enhances male fertility: Wade and Chang, 1995; Hariri *et al.*, 1998). By comparing the performance of infected vs. uninfected females (detection of proximate mutualisms; see Table 4.1), facultative fitness benefits due to *Wolbachia* infection have been found in many arthropod host species, often measured as a direct increase in fecundity or longevity (see Table 4.2; cases 1a and 1b in Figure 4.2). Many of these fitness effects have been measured in the laboratory, but a recent study suggests that *Wolbachia* also increase lifetime reproductive success in the field (Segoli *et al.*, 2013). Frequently, infection exhibits the 'Jekyll and Hyde' type in which *Wolbachia* induce a reproductive phenotype and simultaneously confer some fitness benefit. For example, CI-inducing *Wolbachia* have been found to increase female survival in *Aedes albopictus* (Dobson *et al.*, 2004), and *Drosophila simulans* females infected by male-killing *Wolbachia* produce significantly more daughters than do uninfected females (Uncкless and Jaenike, 2012). These examples illustrate that the clear-cut distinction between parasitic and mutualistic *Wolbachia* is not always possible. In some cases, however, *Wolbachia* increase host fitness without any evident reproductive phenotype ('stand-alone benefit' infection): in the parasitoid wasp *Trichogramma bourarachae*, for example, the only known *Wolbachia* phenotype consists of an increase in fecundity (Vavre *et al.*, 1999b; in most *Trichogramma* species, by contrast, *Wolbachia* induce thelytokous parthenogenesis, see Huigens and Stouthamer, 2003).

Table 4.2: *Wolbachia*-induced facultative fitness benefits.

Fitness benefit Host species	Reproductive manipulation?[a]	Notes	References
Increased fecundity			
ARACHNIDA			
Tetranychus truncatus	CI	perhaps due to the double infection with *Wolbachia* and *Cardinium*	[1]
INSECTA			
Diptera			
Aedes albopictus	CI		[2, 3]
Drosophila innubila	MK		[4]
Drosophila mauritiana	?	due to increased mitotic activity of germline stem cells and decreased apoptosis	[5]
Drosophila melanogaster	—		[6]
Drosophila simulans	CI		[7]
Hemiptera			
Nilaparvata lugens	—		[8]
Hymenoptera			
Nasonia vitripennis	CI	probably due to host genetic background; see Bordenstein and Werren (2000)	[9]
Trichogramma bourarachae	—		[10, 11]
Trichogramma oleae	PI		[12]
Trichogramma pretiosum	PI		[13]
Psocoptera			
Liposcelis tricolor	?		[14]

Table 4.2. Continued

Fitness benefit Host species	Reproductive manipulation?[a]	Notes	References
Increased survival/longevity			
ACARI			
Tetranychus phaselus	—	perhaps due to the interplay between *Wolbachia* and *Cardinium* strains	[15]
INSECTA			
Diptera			
Aedes albopictus	CI		[2, 3, 16]
Aedes polynesiensis	CI		[17]
Culex quinquefasciatus	CI	only in blood fed females	[18]
Drosophila melanogaster	—		[6, 19]
Drosophila melanogaster	?		[20, 21]
Hemiptera			
Bemisia tabaci	?		[22]
Psocoptera			
Liposcelis tricolor	?		[14]
Nutritional provisioning			
INSECTA			
Coleoptera			
Diabrotica virgifera virgifera	CI	due to down-regulation of defense genes in maize host plant; but see Robert *et al.* (2013)	[23]
Diptera			
Drosophila innubila	MK	in low-nutrient environment	[4]
Drosophila melanogaster	CI	in low or high iron environment	[24]

Table 4.2. Continued

Fitness benefit Host species	Reproductive manipulation?[a]	Notes	References
Nutritional provisioning (continued)			
Lepidoptera			
Phyllonorycter blancardella	?	due to cytokinin-mediated induction of 'green-island' phenotype	[25]

[a] CI, cytoplasmic incompatibility; MK, male-killing; PI, parthenogenesis induction; ?, unknown/not reported; —, not detected.

[1] Zhao *et al.* (2013b); [2] Dobson *et al.* (2002); [3] Dobson *et al.* (2004); [4] Uncкless and Jaenike (2012); [5] Fast *et al.* (2011); [6] Fry *et al.* (2004); [7] Weeks *et al.* (2007); [8] Zhang *et al.* (2010); [9] Stolk and Stouthamer (1996); [10] Girin and Boulétreau (1995); [11] Vavre *et al.* (1999b); [12] Silva (1999); [13] Grenier *et al.* (2002); [14] Dong *et al.* (2007); [15] Zhao *et al.* (2013a); [16] Gavotte *et al.* (2010); [17] Brelsfoard and Dobson (2011); [18] Almeida *et al.* (2011); [19] Fry and Rand (2002); [20] Alexandrov *et al.* (2007); [21] Toivonen *et al.* (2007); [22] Xue *et al.* (2012); [23] Barr *et al.* (2010); [24] Brownlie *et al.* (2009); [25] Kaiser *et al.* (2010).

Facultative benefits, both of the 'Jekyll and Hyde' and 'stand-alone' type, could help to explain an unresolved issue concerning the spread of *Wolbachia*: On the one hand, the bacteria infect a major proportion of arthropod species worldwide (see Chapter 2). Horizontal transmission into new host species is likely to be a key factor in shaping this pandemic (see Chapter 3). On the other hand, *Wolbachia* commonly perform poorly after transmission into new hosts. Moreover, reproductive parasitism alone is often insufficient to ensure successful invasion into novel host populations. In the case of CI, for example, there exists a threshold infection frequency below which *Wolbachia* become extinct. Modeling shows that providing a fitness benefit greatly facilitates the invasion and spread of CI-inducing *Wolbachia* in novel hosts, e.g. by removing the invasion threshold (Fenton *et al.*, 2011; see Chapter 5). A recent experimental study suggests that beneficial effects might facilitate *Wolbachia* invasion even if the reproductive phenotype is lost after transmission to the new host. After transfer of a male-killing *Wolbachia* strain from *Drosophila innubila* to *D. simulans*, the recipient host did not suffer from any reproductive manipulation, but instead showed increased longevity. Such immediate beneficial effects could provide the necessary condition for *Wolbachia* to spread from low initial frequencies in novel host species, independently of any reproductive manipulation (Veneti *et al.*, 2012). Note, however, that after *Wolbachia* have overcome the initial obstacles to invasion by providing a fitness benefit, the beneficial effect might attenuate over time (e.g. in the case of host protection; see Section 4.3.3). In the absence of benefits, the bacteria would have to make use of reproductive parasitism to be maintained in the population. Nevertheless, even such temporary beneficial effects are probably important facilitators of *Wolbachia* invasion into new hosts.

Most studies that analyzed *Wolbachia* effects on host fecundity or longevity did not investigate possible mechanisms underlying these effects. Recent work on *Wolbachia*'s role in the female ovaries of *Drosophila mauritiana* might be informative in this respect. Strikingly, *Wolbachia*-infected females produce about four times more eggs than uninfected females (Fast *et al.*, 2011). In *Drosophila*, egg chambers are produced in the germarium, the anterior part of each ovariole that contains the germline stem cells. *Wolbachia* infection in *D. mauritiana* leads to increased mitotic activity of germline stem cells and to decreased apoptosis in the germarium. The combination of both effects results in the fourfold increase in egg production (Fast *et al.*, 2011). Although it is questionable whether such a huge fecundity effect is still beneficial to the host, *Wolbachia* could make use of these mechanisms to a lesser extent in order to enhance host fecundity in a beneficial way.

Moreover, in *Drosophila melanogaster*, *Wolbachia* infection influences the expression level of *chico* (Zheng *et al.*, 2011b), a gene that is involved in life span regulation (Clancy *et al.*, 2001). This could indicate a possible mechanistic basis for *Wolbachia*'s positive effect on longevity in *Drosophila* (Fry and Rand, 2002; Fry *et al.*, 2004).

It is well known that the particular manifestation of mutualistic interactions is often context-dependent (Bronstein, 1994). Accordingly, *Wolbachia*-associated facultative benefits are likely to depend on the environmental conditions experienced by the host. For example, female *Aedes albopictus* larvae that are infected with *Wolbachia* experience higher survivorship under low larval densities, but not under high densities (Gavotte *et al.*, 2010). Additional conditions under which *Wolbachia*-associated benefits appear to be particularly valuable are the presence of pathogens (see Sections 4.3.2 and 4.3.3) and nutritional stress (see Table 4.2; case 1c in Figure 4.2). When exposed to low-nutrient food, infected *D. melanogaster* and *Drosophila innubila* females laid significantly more eggs than uninfected females (Brownlie *et al.*, 2009; Unckless and Jaenike, 2012). A notable case of *Wolbachia*-induced nutritional provisioning was observed in the leaf miner *Phyllonorycter blancardella*. In autumn, *Ph. blancardella* larvae induce 'green islands' in otherwise senescent leaves (Figure 4.1B). These photosynthetically active patches present a nutrient-rich microenvironment to feeding larvae. Interestingly, larvae lose their ability to induce green islands when their mothers were cured of *Wolbachia*, leading to high mortality rates (Kaiser *et al.*, 2010). Probably, *Wolbachia* impact green island formation by manipulating cytokinin levels in the plant, possibly by directly synthesizing the phytohormone. If it could be shown that *Ph. blancardella* on its own (i.e. without *Wolbachia*) has never been able to induce green islands, this would represent a good example of an ultimate mutualism. It has also been suggested that *Wolbachia* may manipulate plant physiology in order to help its herbivorous insect host to cope with plant defense mechanisms. Larvae of the western corn rootworm, *Diabrotica virgifera virgifera*, feed on maize root tissues. A recent microarray study reported that *Wolbachia*-infected larvae induce a down-regulation of maize defense genes compared to their antibiotic-treated counterpart (Barr *et al.*, 2010). However, a follow-up study could not find any evidence of this effect (Robert *et al.*, 2013). Lastly, it is noteworthy that *Wolbachia* might also act as a nutritional mutualist in fungus-growing ants. Workers of the leaf-cutting ant *Acromyrmex octospinosus* cultivate their fungus garden by feeding it with freshly cut leaves and manuring it with faecal droplets. Surprisingly, *Wolbachia* occur extracellularly in the workers' gut lumen and

faecal droplets (Andersen *et al.*, 2012). It is tempting to speculate that *Wolbachia* might contribute to the nutritional function of the faecal droplets in the ant-fungus symbiosis. Taken together, these examples illustrate the role of mutualistic symbionts as 'hidden players' in insect-plant interactions (Frago *et al.*, 2012), but also show that *Wolbachia*'s role in such interactions needs further investigation.

4.3.2 Protection against pathogens: the evidence

The presence of natural enemies is another situation that might reveal possible host benefits provided by *Wolbachia* (case 1d in Figure 4.2). During the last few years, numerous studies have reported that *Wolbachia* infection has an anti-pathogenic effect in the host, for example against several RNA viruses, different *Plasmodium* species, fungi, bacteria, and nematodes. Antiviral effects, in particular, have been observed frequently and across different *Wolbachia* strains, multiple hosts, and diverse viral families (see Table 4.3 and references therein). Some of these studies have aroused great interest, not least because *Wolbachia*'s anti-pathogenic potential might be used as an effective means to control insect-borne human diseases (Kambris *et al.*, 2009; Moreira *et al.*, 2009; Iturbe-Ormaetxe *et al.*, 2011; Blagrove *et al.*, 2012; Mousson *et al.*, 2012; Cook and McGraw, 2010; Caragata *et al.*, 2016; Dutra *et al.*, 2016). The recent upsurge in reports on that topic is also in line with a generally increasing interest in symbiont-mediated protection among arthropod hosts (for reviews, see Haine, 2008; Brownlie and Johnson, 2009; Eleftherianos *et al.*, 2013).

At this point, we put forward a clarification of terminology by distinguishing between 'anti-pathogenic effect' (or 'pathogen interference') on the one hand and 'protection' on the other hand. Symbiont-mediated protection can result from a reduction in pathogen load (resistance), from an increased ability of the host to compensate for negative effects of the pathogen (tolerance), or from a combination of both mechanisms. We define 'protection' as an increase in host fitness as a result of increased resistance and/or tolerance in the presence of pathogens. By contrast, the term 'anti-pathogenic effect' ('interference') is meant to include all cases of increased resistance/tolerance, regardless of whether a corresponding fitness benefit has been demonstrated. While many studies observed an anti-pathogenic effect of *Wolbachia* (mostly based on increased resistance), only some of them have tested for a fitness effect (see Table 4.3). In light of other potential drawbacks (see Section 4.3.3), there remain only a few reports that make a convincing case for *Wolbachia*-mediated host protection

Table 4.3: *Wolbachia*-induced anti-pathogenic effects (pathogen interference).

Host/vector species	Reprod. manip.?[a]	Pathogen	Natural infection?		Fitness effect tested?[b]			
			Wolbachia	Pathogen	*Wolbachia*	Pathogen	*w*MelPop?	Ref.
INSECTA								
Diptera								
Aedes aegypti	CI	Nematode *Brugia pahangi*	No[c]	Yes	No	No	Yes	[1]
	CI	Bacterium *Burkholderia cepacia*	No[c]	?	Yes	Yes	Yes	[2]
	CI	Chikungunya virus	No[c]	Yes	No	No	Yes[d]	[3, 4]
	CI	Dengue virus	No[c]	Yes	Yes[e]	Yes[e]	Yes[d]	[3, 5, 6]
	CI	Bacterium *Erwinia carotovora*	No[c]	?	Yes	Yes[f]	Yes[d]	[1, 2]
	CI	Bacterium *Mycobacterium marinum*	No[c]	No	Yes	Yes	Yes	[2]
	CI	*Plasmodium gallinaceum*	No[c]	No	No	No	Yes	[3]
	CI	Bacterium *Salmonella typhimurium*	No[c]	No	Yes	Yes	Yes[d]	[2]
	CI	Yellow Fever virus	No[c]	Yes	No	No	Yes	[4]

Table 4.3. Continued

Host/vector species	Reprod. manip.?[a]	Pathogen	Natural infection?		Fitness effect tested?[b]			
			Wolbachia	Pathogen	*Wolbachia*	Pathogen	*w*MelPop?	Ref.
Aedes aegypti (continued)		Zika virus	No[c]	Yes	No	No	No	[7, 8]
Aedes albopictus	CI	Chikungunya virus	No[g]	Yes	No	No	No	[9]
	CI	Dengue virus	No[g]	Yes	No	No	No	[10]
	CI	Dengue virus	Yes	Yes	Yes[h]	No	No	[11]
Aedes polynesiensis	CI	Nematode *Brugia pahangi*	No[g]	Yes	Yes[i]	Yes	No	[12]
Anopheles gambiae	?[j]	*Plasmodium berghei*	No[c]	No	No	No	Yes	[13]
	?[j]	*Plasmodium falciparum*	No[c]	Yes	Yes[i]	Yes[h]	Yes[d]	[14]
Anopheles stephensi	CI	*Plasmodium falciparum*	No[c]	Yes	No	No	No	[15]
Culex pipiens	CI	*Plasmodium relictum*	Yes	Yes	Yes	Yes	No	[16]
Culex quinquefasciatus	CI	West Nile virus	Yes	Yes	No	No	No	[17]
Drosophila innubila	MK	Flock House virus	Yes	No	Yes	Yes	No	[18]
Drosophila melanogaster	?	fungus *Beauveria bassiana*	Yes	Yes	Yes	No	No	[19]

Table 4.3. Continued

Host/vector species	Reprod. manip.?[a]	Pathogen	Natural infection?		Fitness effect tested?[b]		*w*MelPop?	Ref.
			Wolbachia	Pathogen	*Wolbachia*	Pathogen		
Drosophila melanogaster (continued)	CI	Chikungunya virus	Yes	No	No	No	No	[17]
	CI	Cricket Paralysis virus	Yes	Yes	Yes	No	No	[20]
	CI	Dengue virus	Yes	No	No	Yes[h]	Yes[d]	[21]
	CI	Drosophila C virus	Yes	Yes	Yes	Yes[k]	Yes[d]	[20, 22]
	CI	Flock House virus	Yes	No	Yes	No	No	[20, 22]
	CI	Nora virus	Yes	Yes	Yes	No	No	[22]
	CI	West Nile virus	Yes	No	No	No	No	[17]
Drosophila simulans	CI	Drosophila C virus	Yes	Yes	Yes	Yes	No	[23]
	CI	Flock House virus	Yes	No	Yes	Yes	No	[23]
Hemiptera								
Cimex lectularius	?	opportunistic bacteria	Yes	Yes	?	?	No	[24]

The column 'Natural infection?' indicates whether the host/vector is naturally infected with *Wolbachia* or the pathogen. The column 'Fitness effect tested?' indicates whether any fitness effects of *Wolbachia* or pathogen infection were tested. The column '*w*MelPop?' indicates whether the laboratory *Wolbachia* strain *w*MelPop was used. Gray shading indicates characters that are not suited for an assessment of *Wolbachia*'s protective potential in the field. For more information see main text.

Table 4.3. Continued

[a] Reproductive manipulation? CI, cytoplasmic incompatibility; MK, male-killing; ?, unknown/not reported.
[b] If yes, then a positive *Wolbachia* effect/negative pathogen effect was found, unless noted otherwise.
[c] Host naturally uninfected.
[d] Not in all experiments.
[e] Only Bian *et al.* (2010) tested for a fitness effect; *Wolbachia* effect was slightly positive, but there was no significant pathogen effect.
[f] Only Ye *et al.* (2013) tested for a fitness effect; pathogen effect was negative.
[g] Cured of its native *Wolbachia* and then transfected with a non-native strain.
[h] No significant effect.
[i] Negative effect.
[j] Only transient somatic infections have been established.
[k] Only Teixeira *et al.* (2008) tested for a fitness effect; pathogen effect was negative.

[1] Kambris *et al.* (2009); [2] Ye *et al.* (2013); [3] Moreira *et al.* (2009); [4] Van den Hurk *et al.* (2012); [5] Bian *et al.* (2010); [6] Walker *et al.* (2011); [7] Aliota *et al.* (2016); [8] Dutra *et al.* (2016); [9] Blagrove *et al.* (2013); [10] Blagrove *et al.* (2012); [11] Mousson *et al.* (2012); [12] Andrews *et al.* (2012); [13] Kambris *et al.* (2010); [14] Hughes *et al.* (2011b); [15] Bian *et al.* (2013); [16] Zélé *et al.* (2012); [17] Glaser and Meola (2010); [18] Uncкless and Jaenike (2012); [19] Panteleev *et al.* (2007); [20] Hedges *et al.* (2008); [21] Rancès *et al.* (2012); [22] Teixeira *et al.* (2008); [23] Osborne *et al.* (2009); [24] L. L. Heaton and M. T. Siva-Jothy, unpublished data.

(e.g. Hedges *et al.*, 2008; Teixeira *et al.*, 2008; Osborne *et al.*, 2009; Zélé *et al.*, 2012; see also Figure 4.1C).

The molecular mechanisms underlying *Wolbachia*-associated anti-pathogenic effects are still unclear. Antiviral activity seems to be more frequent than antibacterial activity, indicating that the underlying mechanisms are independent (Wong *et al.*, 2011; Rottschaefer and Lazzaro, 2012). RNA interference, a major constituent of insect antiviral immunity (Sabin *et al.*, 2010), was shown to be not essential for antiviral effects (Hedges *et al.*, 2012; Rainey *et al.*, 2016; Terradas *et al.*, 2017). Moreover, no effect against a DNA virus has been found so far (Teixeira *et al.*, 2008; Unckless, 2011; Graham *et al.*, 2012). Recently, White *et al.* (2017b) proposed a possible explanation for the RNA virus specificity of *Wolbachia*-associated antiviral effects: RNA viruses, but not DNA viruses, preferentially replicate in the endoplasmic reticulum (ER). Since the authors found *Wolbachia* to be intimately associated with the ER and to dramatically alter its morphology, this might result in interference with viral replication. For a discussion on possible mechanisms underlying *Wolbachia*'s antiviral effects, see also Merkling and van Rij (2013), Sinkins (2013), Rainey *et al.* (2014), and Johnson (2015).

In general, there is good evidence that *Wolbachia* density is correlated to the strength of anti-pathogenic activity (Osborne *et al.*, 2009, 2012; Frentiu *et al.*, 2010; Lu *et al.*, 2012). Consistent with this finding, two major (mutually non-exclusive) hypotheses have been proposed to explain the mechanism of *Wolbachia*-induced pathogen interference. On the one hand, interference may be due to the possibility that both *Wolbachia* and pathogens compete for limited host resources, e.g. cholesterol, which both *Wolbachia* and viruses need for replication (Moreira *et al.*, 2009; Osborne *et al.*, 2009, 2012; Frentiu *et al.*, 2010; Wong *et al.*, 2011; Lu *et al.*, 2012; Caragata *et al.*, 2013). On the other hand, several studies suggest that *Wolbachia* upregulate the host immune response, particularly genes involved in the Toll and the Immune deficiency (Imd) pathway, and that such immune upregulation underlies anti-pathogenic effects (Xi *et al.*, 2008; Moreira *et al.*, 2009; Kambris *et al.*, 2009, 2010; Bian *et al.*, 2010; Pan *et al.*, 2012). However, all of these studies analyzed *Wolbachia* effects in hosts that are either naturally uninfected or infected with a different strain. By contrast, *Wolbachia*-induced anti-pathogenic effects in naturally infected hosts are not associated with immune activation, indicating that upregulation of immune genes (or at least of those in the Toll and Imd pathway) is not required for host protection in the field (Wong *et al.*, 2011; Rancès *et al.*, 2012, 2013; Ferreira *et al.*, 2014) (see next section).

Modeling predicts that host protection will evolve in vertically transmitted symbionts when they compete with horizontally transmitted pathogens in the same host (Lively *et al.*, 2005; Jones *et al.*, 2007, 2011). Accordingly, mechanisms of symbiont-mediated protection can be classified into three categories that correspond to distinct types of interspecies competition from classical ecology (Gerardo and Parker, 2014). *Interference competition* involves the direct impairment of pathogens by symbionts, e.g. by producing toxins that harm pathogens. In the two other forms, competition is indirect. In *exploitative competition*, symbiont and pathogen have overlapping ecological niches and/or compete for the same limited resource; and in *apparent competition*, the symbiont induces a host immune response that is more deleterious to the pathogen than to itself. Notably, most mechanisms that have so far been proposed to underlie *Wolbachia*-mediated anti-pathogenic effects fall into categories of indirect competition, either of the exploitative type (e.g. competition for lipids or for the intracellular ER niche), or of the apparent type (triggering of a host immune response). By contrast, we know of no example showing that *Wolbachia* directly interfere with pathogens to the benefit of the host. Therefore, most anti-pathogenic effects of *Wolbachia* probably do not stem from selection on the symbiont to be beneficial to the host, but are more likely to be a byproduct of other processes (Gerardo and Parker, 2014). Consequently, even if pathogen interference indeed increases host fitness (and thus qualifies as protection; see below), these instances will mostly be 'byproduct benefits', a well-known concept in the literature on the evolution of cooperation (Connor, 1995; Sachs *et al.*, 2004).

4.3.3 Protection against pathogens: a critique

In addition to the fact that pathogen interference is more likely a byproduct than a directly selected trait, there are some further caveats to the experimental findings of *Wolbachia*-associated anti-pathogenic effects and the conclusions that can be drawn from them. Primarily, these caveats relate to the question of whether pathogen interference does occur in nature and, if yes, whether it is associated with a fitness benefit to the host. In other words, does an anti-pathogenic effect actually represent a case of host protection? To answer this question, it is crucial to have a closer look at the ménage à trois between host, pathogen, and *Wolbachia*. With regard to the *Wolbachia*–host relationship, one should ask whether the arthropod species under study is naturally infected with *Wolbachia*, and whether the anti-pathogenic effect is associated with an increase in host fitness. Likewise,

one should examine the studied host-pathogen relationship: is it actually found in nature, and is pathogen presence associated with a decrease in host fitness? We consider each issue in turn.

Does the *Wolbachia*–host relationship exist in nature?

Firstly, several studies that found *Wolbachia*-induced anti-pathogenic effects used the virulent *Wolbachia* strain *w*MelPop (see Table 4.3). This strain was detected in a laboratory strain of *Drosophila melanogaster* and possibly does not exist in nature. It is therefore unclear what these findings tell us about naturally existing symbioses. Secondly, almost all experiments were done using laboratory host strains or even cell lines (Frentiu *et al.*, 2010; Lu *et al.*, 2012). These strains are highly adapted to laboratory conditions which are more benign than those in the field. Again, it is unclear what we can learn about *Wolbachia*-mediated host protection in natural environments. Lastly, and most importantly, a number of studies found pathogen interference in hosts that are naturally uninfected with *Wolbachia* and were only transfected with the symbiont, e.g. the mosquitoes *Aedes aegypti* and *Anopheles gambiae*[1] (Moreira *et al.*, 2009; Kambris *et al.*, 2009, 2010; Bian *et al.*, 2010; Hughes *et al.*, 2011b; Walker *et al.*, 2011). Other reports on anti-pathogenic effects involve hosts that had been cured of their native *Wolbachia* and that were then transfected with a non-native strain (Blagrove *et al.*, 2012, 2013) (see Table 4.3).

Why does the distinction between natural and artificial *Wolbachia* infections matter?

Transfection of *Wolbachia* into naturally uninfected hosts (or into hosts naturally infected with a different *Wolbachia* strain) is likely to be the cause of immune upregulation and thus of the anti-pathogenic effects in these artificially created *Wolbachia*–host associations. By contrast, in many coevolved associations *Wolbachia* infection is not associated with immune upregulation (Bourtzis *et al.*, 2000; Wong *et al.*, 2011; Rancès *et al.*, 2012) and also has no anti-pathogenic effect, but rather is neutral or even 'pro-pathogenic' (see Table 4.4). We also note that even an artificial *Wolbachia* infection can be pro-pathogenic, e.g. by increasing pathogen load (Hughes

[1] The absence of *Wolbachia* in these mosquitoes is probably due to a reciprocal negative interference with *Asaia*, a member of the native microbiota of several mosquito species (Hughes *et al.*, 2014; Rossi *et al.*, 2015). Accordingly, the recent detection of *Wolbachia* sequences in a few *An. gambiae* individuals in Burkina Faso (Baldini *et al.*, 2014) might be explained by an unusual lack of *Asaia* in this mosquito population.

Table 4.4: Naturally occurring *Wolbachia*–host associations in which infection has either no anti-pathogenic effect or even a deleterious ('pro-pathogenic') effect in the presence of pathogens. This neutral/negative effect was proven by comparing pathogen load or host fitness (survival) in the presence vs. absence of *Wolbachia*.

Host/vector species	Pathogen	Ref.
INSECTA		
Diptera		
Aedes aegypti	Chikungunya virus	[1]
	Yellow Fever virus	[1]
Aedes albopictus	Chikungunya virus	[2]
	Dengue virus	[3]
Aedes fluviatilis	*Plasmodium gallinaceum*[a]	[4]
Aedes pseudoscutellaris	nematode *Brugia pahangi*	[5]
Armigeres subalbatus	Japanese encephalitis virus	[6]
Drosophila bifasciata	Drosophila C virus	[7]
	Flock House virus[a]	[7]
Drosophila innubila	*Drosophila innubila* Nudivirus	[8]
Drosophila melanogaster	bacterium *Burkholderia cepacia*	[9]
	bacterium *Erwinia carotovora*	[10]
	Insect Iridescent Virus 6[a]	[11]
	La Crosse virus[a]	[12]
	bacterium *Listeria monocytogenes*	[13]
	bacterium *Mycobacterium marinum*	[9]
	bacterium *Providencia rettgeri*	[13]
	bacterium *Pseudomonas aeruginosa*	[10]
	bacterium *Salmonella typhimurium*	[13]
	bacterium *Serratia marcescens*	[10]
Drosophila neotestacea	nematode *Howardula aoronymphium*	[14]
Drosophila simulans	fungus *Beauveria bassiana*	[15]
	Drosophila C virus	[16]
	bacterium *Erwinia carotovora*	[10]
	Flock House virus[a]	[16]
	parasitoid *Leptopilina heterotoma*	[15]
	bacterium *Pseudomonas aeruginosa*	[10]
	bacterium *Serratia marcescens*	[10]
Lepidoptera		
Spodoptera exempta	*Spodoptera exempta* nucleopolyhedrovirus	[17]

[a] No natural pathogen.

[1] Van den Hurk *et al.* (2012); [2] Mousson *et al.* (2010); [3] Bian *et al.* (2010); [4] Baton *et al.* (2013); [5] Dutton and Sinkins (2005); [6] Tsai *et al.* (2006); [7] Longdon *et al.* (2012); [8] Unckless (2011); [9] Ye *et al.* (2013);

Table 4.4. Continued

[10] Wong *et al.* (2011); [11] Teixeira *et al.* (2008); [12] Glaser and Meola (2010); [13] Rottschaefer and Lazzaro (2012); [14] Jaenike *et al.* (2010); [15] Fytrou *et al.* (2006); [16] Osborne *et al.* (2009); [17] Graham *et al.* (2012).

et al., 2012). Nevertheless, the overall trend of the findings is that a strong immune response and concomitant pathogen interference are frequent in artificial, but rare in natural *Wolbachia*–host associations. In Chapter 6, we will discuss the interactions between *Wolbachia* and the host immune system, and their implications for anti-pathogenic effects, in more detail. A possible conclusion from the overall trend is that anti-pathogenic effects are present only in newly infected hosts and will attenuate through coevolution between host and symbiont (Vavre and Charlat, 2012). Therefore, *Wolbachia*-induced pathogen interference (and associated host protection) might only be a temporary phenomenon. However, even a temporary anti-pathogenic effect in naturally uninfected hosts might be of biological relevance: It could boost *Wolbachia* from very low initial frequencies and thus facilitate invasion into novel host populations (Fenton *et al.*, 2011; see Chapter 5).

Is *Wolbachia* infection associated with a fitness benefit?

In order to demonstrate that *Wolbachia* actually protects its host against a pathogen, the anti-pathogenic effect must be shown to confer a fitness benefit (e.g. increased survival). In many studies, however, the impact of pathogen interference on host fitness was not analyzed at all (see Table 4.3). Furthermore, some of the studies that did test for fitness effects could not find a positive effect (Mousson *et al.*, 2012) or even found a negative one (Hughes *et al.*, 2011b; Andrews *et al.*, 2012). In conclusion, many analyzed *Wolbachia*–host associations are not suited to prove the symbiont's ability to protect its host against pathogens. Lastly, high-density *Wolbachia* infections, which are often associated with strong anti-pathogenic effects (see Section 4.3.2), might shorten host lifespan. Therefore, even if *Wolbachia* infection protects against pathogens, this benefit might be counteracted by the cost of shortened lifespan, possibly causing selection to favour lower levels of protection (Chrostek *et al.*, 2013).

Does the host-pathogen relationship exist in nature?

The second big task in assessing *Wolbachia*'s protective potential is to scrutinize the relationship between host and pathogen. Not all host-pathogen relationships that were examined in the laboratory are actually found in the field. For example, *Wolbachia*-associated effects against the Flock House virus (FHV) were examined in three different *Drosophila* species (Hedges *et al.*, 2008; Teixeira *et al.*, 2008; Osborne *et al.*, 2009; Uncкless and Jaenike, 2012), although FHV is not a natural pathogen of *Drosophila*, but was isolated from a coleopteran (Scotti *et al.*, 1983). Likewise, *Wolbachia*-induced anti-pathogenic effects were observed in mosquito–*Plasmodium* combinations that are not found in nature (Moreira *et al.*, 2009; Kambris *et al.*, 2010). There are more examples of unnatural host-pathogen relationships (see Table 4.3). Tripartite interactions between *Wolbachia*, its host and an unnatural pathogen are probably not well suited to evaluate *Wolbachia*'s protective abilities.

Is pathogen infection associated with a fitness cost?

A last crucial point is to demonstrate a pathogen-induced fitness cost to the host (usually increased mortality). To do so, one has to compare survival rates of pathogen-challenged and unchallenged hosts. Despite this simplicity, only some studies in Table 4.3 used this approach to confirm a pathogen-related fitness cost (Teixeira *et al.*, 2008; Osborne *et al.*, 2009; Andrews *et al.*, 2012; Unckless and Jaenike, 2012; Zélé *et al.*, 2012). Nevertheless, this check is important because not all symbionts commonly referred to as pathogens necessarily reduce host fitness. For example, Teixeira *et al.* (2008) found a *Wolbachia*-induced anti-pathogenic effect against the *Drosophila* Nora virus. However, this virus causes infections that are essentially symptom-free (Habayeb *et al.*, 2009). Even in an artificially created host-pathogen association, there was no significant pathogen effect on host fitness (Rancès *et al.*, 2012). Moreover, it is possible that a fitness cost is only due to the experimental mode of pathogen transmission. In its natural host *Drosophila melanogaster*, for example, Drosophila C virus (DCV) is transmitted by feeding and shows varying pathogenicity (Thomas-Orillard *et al.*, 1995; Hedges and Johnson, 2008). However, when injected into adult flies, DCV turns out to be highly pathogenic, with flies dying within several days after injection. Accordingly, microarray studies that analysed the antiviral response of *D. melanogaster* revealed that only few genes are induced after oral ingestion of DCV, whereas a broad response is triggered

after DCV injection (Roxström-Lindquist *et al.*, 2004; Dostert *et al.*, 2005). Therefore, experiments involving the injection of pathogens might be biased towards higher fitness costs than those that are found in natural host-pathogen relationships. This might be a serious problem because injection of pathogens into adult hosts is a standard transfection procedure and was used in all studies listed in Table 4.3 that consider *Drosophila*–virus relationships. Last we note that a particular strain of DCV (termed DCV_C) even has a beneficial effect on its *Drosophila* host: although DCV_C enhances pre-adult mortality, it increases fecundity and longevity in adult females and might thus confer a net fitness benefit to the host (Thomas-Orillard, 1990; Gomariz-Zilber and Thomas-Orillard, 1993). In this case, an antiviral effect by *Wolbachia* would probably be disadvantageous to the host.

Up to this point, we have considered relationships between a pathogen and its principal host (such as DCV and Nora virus in *Drosophila*). However, the question of whether pathogens induce a fitness cost is particularly controversial in cases where an arthropod species acts as vector of a pathogen, not as its principal host. Indeed, many studies listed in Table 4.3 consider relationships between pathogens and their arthropod vectors, for example mosquito–*Plasmodium* systems or mosquito-borne viruses such as chikungunya and dengue. The degree of pathogen virulence in arthropod vectors is still under debate. Two meta-analyses suggest that, overall, arthropod-borne pathogens reduce the survival of their vectors (Ferguson and Read, 2002; Lambrechts and Scott, 2009). Nevertheless, there are exceptions: For two mosquito–*Plasmodium* combinations in Table 4.3 (*Aedes aegypti–P. gallinaceum* and *Anopheles gambiae–P. falciparum*), Ferguson and Read (2002) found no reduction in vector survival (see also Hughes *et al.*, 2011b). Similarly, there are cases where chikungunya and dengue infection had no influence on vector survival (Bian *et al.*, 2010; Mousson *et al.*, 2010). Therefore, the fact that pathogens do not necessarily impose a fitness cost holds true both for hosts and vectors. However, if there is no fitness cost of pathogen infection, any anti-pathogenic effect induced by *Wolbachia* will probably not be beneficial to the host.

Taken together, we have shown that many reports on *Wolbachia*-associated anti-pathogenic effects fail to prove naturally occurring host protection. While *Wolbachia*-induced pathogen interference is a promising field of research, given its far-reaching implications for disease control, we feel that there is a need to examine more rigorously its significance in the field. We do not claim that *Wolbachia*-induced protection is unimportant in nature; rather, our survey shows that the evidence is limited so far. Future research might easily change the picture.

4.4 *Wolbachia* as obligate mutualists

4.4.1 Overview

It is becoming increasingly clear that several arthropod species cannot survive or reproduce when their *Wolbachia* symbionts are removed (see Table 4.5). In such cases of evolved dependence (transition 2 in Figure 4.2), hosts have adapted to the presence of *Wolbachia* (De Mazancourt *et al.*, 2005). For example, the latter might have evolved to provide some vital component of a host developmental or reproductive process. Subsequent relaxed selection on host genes for this trait would allow for the accumulation of mutations in these genes. Once the host has lost the ability to provide the vital function on its own, *Wolbachia* could permanently take over control of the corresponding process. Such sheltering of deleterious mutations has been observed in *Drosophila melanogaster* where *Wolbachia* infection suppresses sterility in *Sex-lethal* (*Sxl*) mutants and lethality of *chico* mutants, respectively (Starr and Cline, 2002; Clark *et al.*, 2005). Similarly, infection also rescues female fertility in *bag of marbles* (*bam*) mutants (Flores *et al.*, 2015). Since all genes are involved in *D. melanogaster* oogenesis (*Sxl*, in addition, is the master regulator of sex determination in this species), these observations indicate that dependence on *Wolbachia* might frequently be associated with the ability of the symbiont to interfere with key host reproductive processes, such as oogenesis and sex determination (see Sections 4.4.2–4.4.4).

It is also conceivable that hosts become dependent on their protective symbionts because the latter reduce selection on hosts to maintain their own defense mechanisms (Gerardo and Parker, 2014; Ford and King, 2016). Hence, the presence of defensive symbionts may slow down the evolution of host resistance genes, as was recently shown in *Wolbachia*-infected *D. melanogaster* (Martinez *et al.*, 2016). This may potentially lead to some form of 'immunological dependence'. Possibly, the loss of immune genes in honey bees and pea aphids (Evans *et al.*, 2006; Gerardo *et al.*, 2010) can also be explained by protective symbionts partially taking over host defense.

The takeover of some host function by *Wolbachia* is likely to be typical of the initial steps towards obligate mutualism (case 2a in Figure 4.2, see Section 4.4.3; another evolutionary path to obligate mutualism involves compensatory evolution in the host; see Sections 4.4.2 and 4.4.4). However, some authors have refrained from classifying such relationships as mutualisms and prefer the term 'obligatory parasitism' because *Wolbachia* does not provide any additional new function, but only ensures that pre-exisiting pro-

Table 4.5: Evolved host dependencies upon *Wolbachia*.

Defects in aposymbiotic females Host species	Reproductive manipulation?[a]	Notes	References
Female sterility (oogenesis defects)			
ENTOGNATHA			
Folsomia candida	PI	Via facilitation of parthenogenesis; see Hafer and Pike (2010)	[1, 2]
INSECTA			
Coleoptera			
Coccotrypes dactyliperda	?	Caused by *Wolbachia* or *Rickettsia* (or both)	[3]
Lissorhoptrus oryzophilus	?		[4]
Otiorhynchus sulcatus	?		[5]
Diptera			
Drosophila paulistorum	CI		[6]
Exorista sorbillans	?		[7]
Hemiptera			
Cimex lectularius	?	Via supply of B vitamins	[8]
Hymenoptera			
Asobara tabida	—		[9]
Lethality			
INSECTA			
Lepidoptera			
Eurema mandarina (former *Eurema hecabe*)	FE	Via interference with sex-specific-splicing of *doublesex*	[10]

Table 4.5. Continued

Defects in aposymbiotic females Host species	Reproductive manipulation?[a]	Notes	References
Lethality (continued)			
Ostrinia furnacalis	MK	Via interference with dosage compensation	[11]
Ostrinia scapulalis	MK	Via interference with dosage compensation	[12]

[a] CI, cytoplasmic incompatibility; FE, feminization; MK, male-killing; PI, parthenogenesis induction; ?, unknown/not reported; —, not detected.

[1] Pike and Kingcombe (2009); [2] Timmermans and Ellers (2009); [3] Zchori-Fein *et al.* (2006); [4] Chen *et al.* (2012); [5] Son *et al.* (2008); [6] Miller *et al.* (2010); [7] Puttaraju and Prakash (2009); [8] Hosokawa *et al.* (2010); [9] Dedeine *et al.* (2001); [10] Kageyama *et al.* (2017); [11] Fukui *et al.* (2015); [12] Sugimoto *et al.* (2015)

cesses function properly, thus rendering the host incapable of independence (Dedeine *et al.*, 2003). The conflict can be resolved by the disambiguation put forward by De Mazancourt *et al.* (2005): symbioses in which the host requires *Wolbachia* are classified as proximate mutualisms (the host derives a benefit from *Wolbachia*'s presence) and as obligate mutualisms (arising from evolved dependence), but usually not as ultimate mutualisms (there is no additional benefit) (see Table 4.1). The loss of a trait whose function is taken over by an ecological partner has also been termed 'compensated trait loss'; its importance for the evolutionary stability of ecological interactions has been recognized only recently (Ellers *et al.*, 2012).

Just as with facultative benefits, some obligate mutualisms are associated with reproductive manipulation, while others are not. In addition, there are certain kinds of obligate mutualism that could only arise because of the reproductive phenotype. We present in detail a case study for each of the three scenarios. Here, a main focus will be on the question how these dependencies evolved, both in evolutionary and developmental terms. This allows us to highlight both commonalities and differences between different forms of dependence, but also between mutualistic and parasitic *Wolbachia* phenotypes.

4.4.2 Dependence without a reproductive phenotype: the case of *Asobara tabida*

The first report of an arthropod species being completely dependent on its *Wolbachia* symbiont comes from the parasitoid wasp *Asobara tabida* (Figure 4.1D). Here, females that are cured of their *Wolbachia* (i.e. aposymbiotic females) fail to produce mature oocytes (Dedeine *et al.*, 2001). This case has risen to prominence even beyond the *Wolbachia* community and is frequently used to illustrate the role of symbiosis in animal development (Gilbert *et al.*, 2010; McFall-Ngai *et al.*, 2013). *A. tabida* is infected with three distinct *Wolbachia* strains, only one of which is required for oogenesis, whereas the two other strains induce CI (Dedeine *et al.*, 2004). No other species of the genus *Asobara* depends on *Wolbachia* for oogenesis, suggesting that the dependence in *A. tabida* has evolved recently (Dedeine *et al.*, 2005b). Although the oogenesis defects in *A. tabida* resemble those in *Sxl* female-sterile mutants in *Drosophila melanogaster* (Starr and Cline, 2002; see Section 4.4.1), recent findings suggest that dependence in *A. tabida* did not evolve by *Wolbachia* simply taking over control of some host function. In the following, we summarize what we know about the mechanisms underlying this dependence, and how it might have evolved.

The failure of oogenesis in aposymbiotic females has been shown to be due to extensive apoptosis of nurse cells in mid-stage egg chambers (Pannebakker *et al.*, 2007). Apoptosis is an essential part of insect oogenesis. At the end of *Drosophila* oogenesis, developmental apoptosis of nurse cells occurs after their cytoplasmic contents have been transferred to the oocyte (a process called dumping). In addition, cell death may be triggered at distinct checkpoints during early and mid-oogenesis in response to adverse stimuli (McCall, 2004). The results by Pannebakker *et al.* (2007) suggest that *Wolbachia* is necessary for egg chambers to pass the mid-oogenesis checkpoint by preventing apoptosis. The authors outline a coevolutionary scenario in which *A. tabida* responds to infection with apoptosis, which is then suppressed by *Wolbachia*, and the host in turn compensates for suppression by further increasing apoptosis because it is essential to complete oogenesis (Pannebakker *et al.*, 2007). This scenario is based on the pleiotropic role of programmed cell death in development and immunity (Vavre *et al.*, 2008), and there is good empirical support for it. Although evidence for apoptosis as a host defense against *Wolbachia* is rather scant, apoptotic cell death is a common immune response to viral infections among insects (Clarke and Clem, 2003). Moreover, autophagic cell death was recently shown to regulate *Wolbachia* populations in mosquito and *D. melanogaster* cell lines (Voronin *et al.*, 2012). Further support for the involvement of cell death pathways in the insect immune response comes from the fact that bacterial suppression of such pathways is widespread (Faherty and Maurelli, 2008). Strikingly, a native *Wolbachia* strain in *Drosophila mauritiana* is able to significantly decrease apoptosis in the female ovary (Fast *et al.*, 2011). So far, there are two candidate genes whose expression might be manipulated by *Wolbachia* in order to decrease apoptosis. Both *chico* and *lola* are involved in the apoptotic pathway of the *Drosophila* mid-oogenesis checkpoint (McCall, 2004; Bass *et al.*, 2007). In a recent gene expression analysis of *D. melanogaster* larval testes, expression of both genes was found to be altered in *Wolbachia*-infected flies compared to uninfected flies (Zheng *et al.*, 2011b). Moreover, in *chico* mutant lines infected with *Wolbachia*, symbiont removal results in complete lethality of homozygous mutants, although this *Wolbachia* effect appears to be not directly linked to *chico* (Clark *et al.*, 2005). These results suggest that *Wolbachia* might interfere with apoptosis in the *Drosophila* ovary by targeting *chico* and/or *lola*. Lastly, the *Wolbachia* surface protein (WSP) of nematode-associated *Wolbachia* inhibits apoptosis in human neutrophils (Bazzocchi *et al.*, 2007). Taken together, these findings indicate that *Wolbachia* could directly manipulate apoptotic pathways in *A. tabida* ovaries.

There is also evidence that *Wolbachia* can act indirectly on host apoptotic processes. Kremer *et al.* (2009b) showed that *Wolbachia* interferes with iron metabolism in *A. tabida*, particularly with the expression of ferritin, a protein involved in iron storage and oxidative stress regulation. The authors suggest that *Wolbachia*, which is known to induce oxidative stress in another host system (Brennan *et al.*, 2008), can thus disrupt cellular physiology, including apoptosis. Regardless of whether the effect is direct or indirect, *Wolbachia*-induced suppression of apoptosis in the ovaries should select for increased apoptotic signaling in the host to enable developmental apoptosis of nurse cells after dumping. Thus, the host should evolve some form of tolerance (a strategy to reduce fitness costs of infection; see also Section 4.4.5) to compensate for the harmful effects of the symbiont. In the absence of *Wolbachia*, this compensatory evolution would result in excessive apoptosis and therefore inhibition of oogenesis, rendering *A. tabida* completely dependent on its symbiont (Aanen and Hoekstra, 2007). Is there any molecular evidence for such compensatory evolution in *A. tabida*?

Interestingly, there is a high level of intraspecific variation in *A. tabida* regarding the degree to which wasps depend on *Wolbachia* for oogenesis. Whereas most aposymbiotic females are unable to produce mature oocytes, there are *A. tabida* lines in which cured females can produce some eggs; however, larvae hatched from these eggs die early during development (Dedeine *et al.*, 2005b). Very few eggs laid by aposymbiotic females even develop to adulthood; however, lines derived from these individuals are unable to be maintained (Kremer *et al.*, 2010). Therefore, despite considerable variation in the phenotype of aposymbiotic females (also termed the 'ovarian phenotype'), in no case are viable offspring produced, implying that the ovarian phenotype cannot be subject to direct selection. In an elegant work, Kremer *et al.* (2010) offer a possible explanation for this puzzle. They propose that the ovarian phenotype could be indirectly selected if it is correlated with traits that are under direct selection. The authors then argue that host mutations that compensate for the anti-apoptotic effects of *Wolbachia* are likely to be selected for. Indeed, they could show that the ovarian phenotype is correlated with the expression of genes that are involved in iron metabolism and oxidative stress control, e.g. ferritin (Kremer *et al.*, 2010, 2012). Exactly those genes are manipulated by *Wolbachia* to interfere with host apoptosis and therefore are likely to be used by *A. tabida* to counteract the symbiont's harmful effects. Moreover, these differences in gene expression are also present in *Wolbachia*-infected females (which do produce viable offspring), making direct selection possible (Kremer *et al.*, 2010). Thus, these findings strongly suggest that complete

dependence of *A. tabida* on its symbiont is the result of compensatory evolution in the host.

The ability of *Wolbachia* to interfere with host apoptosis and iron metabolism pathways is not restricted to the *A. tabida* mutualism, but has also been observed in other relationships, both mutualistic and parasitic. This fact allows us to compare the mechanisms that are used by *Wolbachia* to interact with the host in different symbiotic relationships. Kremer *et al.* (2009b) showed that *Wolbachia* affect iron metabolism not only in the obligate mutualism with *A. tabida*, but also in the facultative parasitism with *Drosophila simulans* (where *Wolbachia* induces CI) and in *Aedes aegypti* cells. In a facultative mutualism with *D. melanogaster*, *Wolbachia* has a positive fecundity effect in low- or high-iron environments, again suggesting that the symbiont is involved in iron hoemostasis (Brownlie *et al.*, 2009). Furthermore, interference with both host iron metabolism and apoptosis has been suggested to be involved in the *Wolbachia*–nematode mutualism. The biosynthetic pathway of heme (which plays a central role in iron metabolism) is absent in the nematode *Brugia malayi* (Ghedin *et al.*, 2007), whereas its *Wolbachia* symbiont has all but one gene for heme biosynthesis, suggesting that worms depend on acquiring heme from their symbionts (Foster *et al.*, 2005). In addition, it has recently been shown that depletion of *Wolbachia* from *B. malayi* leads to extensive apoptosis in the adult germline, which offers another potential basis for this mutualistic symbiosis and, moreover, mirrors the situation in *A. tabida* (Landmann *et al.*, 2011). These results indicate that parasitic and mutualistic *Wolbachia* use the same molecular mechanisms to interact with their hosts.

Taken together, the dependence of *A. tabida* on *Wolbachia* nicely illustrates the role of tolerance or compensatory evolution in the transition from parasitism to mutualism (Aanen and Hoekstra, 2007; Edwards, 2009) (case 2b in Figure 4.2). Moreover, although probably no ultimate mutualism, it serves as a prime example of evolved dependence and obligate mutualism (De Mazancourt *et al.*, 2005; Edwards, 2009).

4.4.3 Dependence associated with a reproductive phenotype: manipulation of sex determination in lepidopterans

Here we present examples of obligate mutualisms where dependence has evolved concomitantly with reproductive manipulation. In the adzuki bean borer, *Ostrinia scapulalis*, *Wolbachia*-infected females produce all-female broods, indicative of a sex-ratio distorting phenotype. Such all-female broods were shown to be due to the death of genetic males, suggesting

a male-killing effect of *Wolbachia*. Unexpectedly, however, cured females give rise to all-male progeny, which is due to the death of genetic females (Kageyama and Traut, 2004). This finding indicates that *O. scapulalis* has evolved some form of dependence on its *Wolbachia* symbionts as the latter appear to be required for female development. Moreover, the sex-specificity of death suggests that *Wolbachia* somehow interferes with the sex determination system of its host. Indeed, recent studies show that *Wolbachia*'s manipulation of *O. scapulalis* sex determination plays a crucial role in both the sex-ratio distorting and the dependence phenotype.

In many insect species, it is the chromosomal constitution that serves to start the sex determination pathway. As in most lepidopterans, *O. scapulalis* has a female heterogametic sex chromosome system, i.e. females are heterogametic (ZW), and males are homogametic (ZZ) (Kageyama and Traut, 2004). The gene *doublesex* (*dsx*) is the conserved master switch at the bottom of the insect sex determination cascade. Due to sex-specific splicing, *dsx* exists as a male or a female isoform, which starts male- or female-specific development, respectively (Sánchez, 2008). In female embryos of the lepidopteran *Bombyx mori*, a feminizing factor (*Fem*) on the W chromosome silences the transcripts of a masculinizing factor (*Masc*) on the Z chromosome, leading to female-specific splicing of *dsx*. In male embryos, *Masc* is responsible both for male-specific splicing of *dsx* and for dosage compensation, a process necessary to adjust the expression levels of Z-linked genes, jointly leading to male development (Kiuchi *et al.*, 2014). Recently, a *dsx* homologue was identified in *O. scapulalis*, which was termed *Osdsx* (Sugimoto *et al.*, 2010). Interestingly, in *Wolbachia*-infected individuals, the female-type *Osdsx* is expressed irrespective of the genetic sex. By contrast, in individuals that have been cured of infection, the male-specific splice form is expressed irrespective of the genetic sex (Sugimoto and Ishikawa, 2012). Death occurs if there is a mismatch between genetic sex (ZW or ZZ) and phenotypic sex (male- or female-specific *Osdsx*). Sugimoto *et al.* (2015) showed that *Wolbachia*-associated sex-specific death (male-killing when present, female-killing when removed) is due to a failure of dosage compensation. The expression of the female-specific *Osdsx* in genetic males suggests that *Wolbachia* carries a *Fem*-like feminizing factor. This would lead to *Masc* silencing, defective dosage compensation and abnormally high expression of Z-linked genes. Moreover, the fact that genetic females develop a male phenotype in the absence of *Wolbachia* suggests that the W chromosome lacks the *Fem*-like factor. Consequently, *Masc* is not silenced, leading to abnormally low expression of Z-linked genes.

Taken together, the findings by Sugimoto and Ishikawa (2012) and Sugimoto *et al.* (2015) suggest that *Wolbachia*'s feminizing factor performs the function of the *Fem* gene that has been lost from the W chromosome in *O. scapulalis* (case 2a in Figure 4.2). The pattern of bidirectional sex-specific death associated with *Wolbachia* infection was also observed in the closely related species *Ostrinia furnacalis* (Sakamoto *et al.*, 2007). Recently, it was shown that male death in *O. furnacalis* is based on the same mechanism as in *O. scapulalis*, i.e. repression of *Masc* and subsequent failure of dosage compensation (Fukui *et al.*, 2015). Therefore, this species is likely to have evolved essentially the same dependence on *Wolbachia* as *O. scapulalis*. Note that, in these cases, bacterial disturbance of host sex determination is facilitated by female heterogamety: since the female-specific W chromosome is co-inherited with *Wolbachia*, W-specific functions can be lost and substituted by the symbiont. This is not possible in hosts with male heterogamety.

In the female-heterogametic butterfly *Eurema mandarina* (former *Eurema hecabe*), the production of all-female broods in *Wolbachia*-infected populations was long thought to be due to the feminization of genetic males (ZZ) (Hiroki *et al.*, 2002; Narita *et al.*, 2007). However, this notion was challenged by the finding that infected females have only one Z chromosome that is paternally inherited, suggesting that meiotic drive excludes the maternal Z and thus prevents the formation of ZZ zygotes (Kern *et al.*, 2015). Subsequent work by Kageyama *et al.* (2017) corroborated that infected females have a Z0 genotype and that *Wolbachia* cause both feminization of Z0 individuals and meiotic drive. Antibiotic treatment of infected females results in the production of both Z0 and ZZ eggs (as expected in the absence of meiotic drive). However, only very few of these Z0 eggs survive in the absence of feminizing effects of *Wolbachia*. Moreover, antibiotic treatment at larval stages leads to intersexual phenotypes and high pupal mortality (Narita *et al.*, 2007; Figure 4.1E). Kageyama *et al.* (2017) could show that intersexes have a Z0 genotype, but express both the male- and female-specific splicing product of *E. mandarina dsx (Emdsx)*. These findings suggest that *Wolbachia* are required for female development of Z0 individuals, and that death might be due to a failure of dosage compensation, as evident in *Ostrinia*. Kageyama *et al.* (2017) conceive the following evolutionary scenario to account for the observed effects. *Wolbachia*-induced feminization is lethal to ZZ males, probably also because of improper dosage compensation. In infected ZW females, the female-determining function of the W chromosome became redundant and was taken over by the bacteria, leaving the hosts dependent on their symbionts for female development. In

contrast to *Ostrinia*, however, the W chromosome was lost completely in *E. mandarina*. After the loss of the W chromosome, *Wolbachia* evolved an additional property: the ability to induce meiotic drive. By preventing the production of ZZ zygotes (which are doomed when infected), the bacteria gain the advantage of being transmitted by all zygotes, and not only by half of them. In sum, these findings show that male-killing and feminization can be closely intertwined and that host sex determination might be particularly prone to microbial manipulation and to subsequent evolution of dependence.

4.4.4 Dependence through a reproductive phenotype: parthenogenesis–inducing *Wolbachia* and their hosts

In haplodiploid species, *Wolbachia*-induced thelytokous parthenogenesis can lead to the complete elimination of males in populations where infection has gone to fixation. These populations consist entirely of infected females which reproduce parthenogenetically; sexual reproduction is no longer present. The loss of sexual functionality makes *Wolbachia* an obligate mutualist for daughter production in all-female populations. Such obligate mutualism between arthropod hosts and their PI-*Wolbachia* has evolved in numerous haplodiploid species, mainly hymenopterans, but also in the mite species *Bryobia praetiosa* and in the thysanopteran *Franklinothrips vespiformis* (see Table 4.6; Figure 4.1F). In what follows, we summarize evidence of how this dependence could evolve.

Interestingly, the lack of sex in fixed populations is due to a complete loss of sexual function in females, but not in males. This was shown in host species in which both fixed (asexual) and uninfected (sexual) populations exist. Males can be derived from fixed populations by antibiotic treatment. When such males are mated with females from sexual populations, the latter successfully fertilize the eggs. However, when females from fixed populations are exposed to males, they do not fertilize their eggs (Pijls *et al.*, 1996; Kremer *et al.*, 2009a; Russell and Stouthamer, 2011). In some cases, the morphological or physiological aberrations underlying the failure of fertilization are known: in *Muscidifurax uniraptor*, females lack a spermathecal muscle, and in *Trichogramma cordubensis*, females are not attractive to conspecific males, possibly due to lacking pheromone production (Gottlieb and Zchori-Fein, 2001; Silva and Stouthamer, 1997).

Several hypotheses have been proposed to explain the female-specific decay of sexual function in fixed populations and the concomitant evolution of dependence on PI-*Wolbachia*. The 'costly female trait' hypothesis says that

Table 4.6: Loss of sexual function due to infection with PI-*Wolbachia*.

Host species	References
ARACHNIDA	
Bryobia praetiosa	Weeks and Breeuwer (2001)
INSECTA	
Hymenoptera	
Aphytis diaspidis	Zchori-Fein *et al.* (1995)
Aphytis lingnanensis	Zchori-Fein *et al.* (1995)
Apoanagyrus diversicornis	Pijls *et al.* (1996)
Asobara japonica	Kremer *et al.* (2009a)
Encarsia formosa	Zchori-Fein *et al.* (1992)
Eretmocerus mundus	De Barro and Hart (2001)
Gronotoma micromorpha	Arakaki *et al.* (2001a)
Leptopilina clavipes	Pannebakker *et al.* (2005)
Muscidifurax uniraptor	Gottlieb and Zchori-Fein (2001)
Telenomus nawai	Arakaki *et al.* (2000), Jeong and Stouthamer (2005)
Trichogramma cordubensis	Silva and Stouthamer (1997)
Trichogramma pretiosum	Russell and Stouthamer (2011)
Thysanoptera	
Franklinothrips vespiformis	Arakaki *et al.* (2001b)

in the absence of sex, costly female traits involved in sexual reproduction, e.g. pheromone production, will be selected against (Pijls *et al.*, 1996). The 'functional virginity' hypothesis proposes that the female-biased sex ratio in populations with a spreading PI-*Wolbachia* infection selects for mutations that increase the production of males in order to restore the optimal sex ratio. In haplodiploid species, this is achieved by lowering the fertilization rate. Thus, any mutation occurring in females that reduces the fertilization frequency will be selected, including even 'virginity' alleles which disable any trait required for successful sexual reproduction (Huigens and Stouthamer, 2003; Jeong and Stouthamer, 2005). Recent modeling favours the latter hypothesis: Stouthamer *et al.* (2010) showed that selection for lower fertilization rates ultimately results in the population becoming fixed for both the PI-*Wolbachia* infection and the virginity alleles. Once infection is fixed, mutations interfering with costly female traits will spread (Pijls *et al.*, 1996), and other genes involved in reproduction will accumulate mutations both in males and females (Carson *et al.*, 1982). Thus, the nucleo-cytoplasmic conflict over sex ratio is eventually resolved

by an irreversible loss of sexual reproduction (Stouthamer *et al.*, 2010). In line with theory, putative 'functional virginity' loci responsible for the loss of female sexual function have been identified in *Telenomus nawai* and *Trichogramma pretiosum* (Jeong and Stouthamer, 2005; Russell and Stouthamer, 2011). These findings show that selection can promote the evolutionary transition to obligate asexuality, associated with complete dependence on *Wolbachia* (King and Hurst, 2010).

The evolution of obligate mutualism involving PI-*Wolbachia* demonstrates another example of how dependence can result from compensatory evolution (tolerance) in the host (case 2b in Figure 4.2), which, in this case, involves decreasing the fertilization rate to counteract the female-biased sex ratio. Exactly this host compensatory mechanism has also evolved in the haplodiploid mite *Tetranychus urticae*. Although not due to parthenogenesis induction, *Wolbachia*-infected *T. urticae* females produce more female-biased sex ratios than cured females. Interestingly, it is the sex ratio produced by infected females that best approaches the optimal sex ratio (which, due to local mate competition, is female-biased in *T. urticae*) (Vala *et al.*, 2003). Why is the sex ratio produced by cured females less than optimal? Vala *et al.* (2003) propose that, in response to the *Wolbachia*-induced shift in sex ratio (which initially was too female-biased), *T. urticae* decreased the fertilization rate in order to restore the optimal sex ratio. However, this compensatory mechanism is costly in the absence of *Wolbachia* because then the sex ratio is too male-biased. Again, obligate mutualism is a likely outcome of such compensatory evolution.

If the functional virginity hypothesis is correct, it has some interesting implications. Firstly, the theoretical finding by Stouthamer *et al.* (2010) that any allele that lowers the fertilization rate will become fixed nicely corroborates the prediction that any tolerance gene should be driven to fixation by natural selection (Roy and Kirchner, 2000). Secondly, it is only selection on the host that eventually leads to the evolution of dependence on PI-*Wolbachia*. This stands in contrast to other cases of evolved dependence in which selection on *Wolbachia* causes, or at least contributes to, the dependence phenotype (in addition to selection that gave rise to the reproductive phenotype itself). This issue can be exemplified by two closely related wasp species that both depend on *Wolbachia* for reproduction, but for completely different reasons. In *Asobara tabida*, dependence on *Wolbachia* for oogenesis could emerge ultimately because the symbiont evolved the ability to interfere with host apoptotic processes (see Section 4.4.2). In *A. japonica*, by contrast, infection with PI-*Wolbachia* has selected for lower fertilization rates and, eventually, led to the decay of

sexual function in females and thus dependence (Kremer *et al.*, 2009a). Hence, although compensatory mechanisms underlie the dependence in both *Asobara* species, the evolutionary trajectories leading there are distinct.

4.4.5 Resistance, tolerance, and dependence

Resistance and tolerance are two distinct host strategies to cope with infection. Whereas resistance aims at limiting the infection, tolerance does not reduce the infection itself, but limits its fitness consequences (Roy and Kirchner, 2000). This review shows that tolerance to *Wolbachia* has evolved repeatedly in arthropod hosts. By contrast, resistance alleles have rarely been found in host species, although there is strong selection to counteract *Wolbachia*'s reproductive parasitism (Charlat *et al.*, 2007; Koehncke *et al.*, 2009). Why might host resistance to *Wolbachia* be rare? A possible reason is that, once resistance has led to the loss of infection, costly but redundant resistance alleles are likely to be lost as well. While this conjecture does not rule out that resistance itself evolves frequently (transition 8 in Figure 4.2), there might be circumstances in which resistance is not the best strategy of responding to *Wolbachia* infection. Here we show two barriers to the evolution of host resistance, both of which are associated with *Wolbachia* mutualisms. Obviously, resistance should not evolve if *Wolbachia* confers a net fitness benefit to the host ('fitness benefit' barrier to resistance; see Chapter 5). A second barrier to resistance is closely linked to the evolution of tolerance in response to *Wolbachia* infection. This can be illustrated by any host compensatory mechanism, e.g. the decrease in fertilization rate to counteract the symbiont-induced female-biased sex ratio (see Section 4.4.4). Such compensatory mechanisms leading to host tolerance are costly in the absence of infection. If these costs are too high, there will be selection on females to foster vertical transmission of their symbiont (Law and Dieckmann, 1998). Thus, selection for tolerance favours the evolution of dependence (obligate mutualism) (Roy and Kirchner, 2000; Aanen and Hoekstra, 2007; Edwards, 2009; see Sections 4.4.2 and 4.4.4). Once the host depends on *Wolbachia*, resistance is no longer an option ('dependence' barrier to resistance). On these grounds, both beneficial effects of, and tolerance to, *Wolbachia* can hinder the evolution of host resistance.

4.5 Antibiotic treatment and *Wolbachia* effects: a critical note

Most experimental approaches to identify mutualisms have investigated the performance of a given host in the presence and absence of its symbiont (Douglas and Smith, 1989). In the case of *Wolbachia*, infected hosts are cured of the infection by antibiotic treatment to compare the performance of cured individuals with that of their untreated counterparts. Usually, the broad-spectrum antibiotic tetracycline is used (Li *et al.*, 2014). It is implicitly assumed that tetracycline treatment has no other effect than removing *Wolbachia*. However, this is not always the case. Other symbionts (e.g. gut bacteria) are likely to be removed as well. Therefore, effects attributed to *Wolbachia* might in fact be caused by other bacteria. This can be exemplified by the role of symbionts in reproductive isolation in *Drosophila melanogaster*. Koukou *et al.* (2006) eliminated *Wolbachia* from *D. melanogaster* cage populations by tetracycline treatment and found that the preexisting sexual isolation between populations was reduced by about 50%. However, this effect could be due to any tetracycline-sensitive bacteria of the *D. melanogaster* microbiota. Indeed, recent results suggest that, rather than *Wolbachia*, *Lactobacillus* bacteria are responsible for the mating preference in *D. melanogaster* (Sharon et al. 2010).

Another largely disregarded effect of tetracycline concerns mitochondrial metabolism and mitochondrial DNA (mtDNA) density. Tetracycline works by blocking the 30S subunit of prokaryotic ribosomes, thus inhibiting translation and protein synthesis. Descending from bacterial ancestors, mitochondria have bacteria-type ribosomes, and thus tetracycline also inhibits mitochondrial protein synthesis (Zhang *et al.*, 2005). Indeed, in *Drosophila simulans*, tetracycline treatment reduces mitochondrial efficiency and probably leads to decreased ATP production. This could have a direct influence on fecundity or longevity, which may easily be confused with a *Wolbachia* effect (Ballard and Melvin, 2007). Moreover, tetracycline treatment causes an increase in mtDNA copy number in *Wolbachia*-uninfected fly lines, which is probably a consequence of tetracycline-induced inhibition of mtDNA translation. In infected flies, by contrast, tetracycline has no effect on mtDNA copy number because the presence of *Wolbachia* dilutes the concentration of the antibiotic in the mitochondria (Ballard and Melvin, 2007). This differential effect of tetracycline on infected and uninfected flies might impair experimental controls in the laboratory. In the field, on the other hand, the antibiotic-diluting effect of *Wolbachia* will be beneficial

only if the host is exposed to antibiotics in its environment, and if this antibiotic is somehow detrimental to host fitness. Finally, the effects of tetracycline on mitochondria in *D. simulans* were observed two generations after treatment (see also Zeh *et al.*, 2012). In light of these findings, it is essential that researchers carefully control for antibiotic effects other than *Wolbachia* removal. Otherwise, *Wolbachia* might be held responsible for effects that either are caused by other symbionts or actually do not exist in the field.

4.6 The evolutionary fate of *Wolbachia*–arthropod mutualisms

What will happen to *Wolbachia* that have evolved a mutualistic association with their arthropod hosts? The question of whether mutualistic relationships are evolutionarily stable or whether transitions between mutualism and parasitism occur is an ongoing debate in evolutionary biology (Moran and Wernegreen, 2000; Sachs and Simms, 2006; Sachs *et al.*, 2011b). In the following, we briefly present possible evolutionary outcomes of mutualism in *Wolbachia*–arthropod associations (Figure 4.2).

Facultative mutualisms that are based on environment-dependent fitness benefits might easily break down if the environment changes so that the cost-benefit ratio (i.e. the net effect on host fitness) becomes unfavourable. In the case of 'Jekyll and Hyde' infections, this would leave *Wolbachia* as pure reproductive parasites (transition 3 in Figure 4.2). Moreover, such transitions from mutualism to reproductive parasitism probably are particularly relevant in the context of temporary benefits which help *Wolbachia* to invade a population. After such benefits have helped to overcome the invasion threshold, they might attenuate over time so that *Wolbachia* would have to rely on a reproductive manipulation to be maintained. Alternatively, facultative mutualisms might break down in 'stand-alone benefit' infections. In this case, *Wolbachia* would be prone to extinction in the absence of any mechanism to maintain them in the population (transition 4 in Figure 4.2). In sum, facultative *Wolbachia* mutualisms seem to be relatively unstable and, owing to shifts in the cost-benefit ratio, might easily switch to parasitism. Given that there is also good evidence for the reverse switch (from parasitism to mutualism, which is the subject matter of this Chapter), these findings together indicate that the two forms of symbiosis are often dynamic in *Wolbachia*–arthropod associations.

Facultative mutualisms might become obligate if dependence is evolving

in the context of a developmental or reproductive pathway that is already manipulated by *Wolbachia* to provide the facultative benefit (transition 5 in Figure 4.2). For example, *Wolbachia*'s ability to interfere with iron metabolism is presumably used both in the facultative mutualism with *Drosophila melanogaster* and in the obligate mutualism with *Asobara tabida* (Brownlie *et al.*, 2009; Kremer *et al.*, 2009b; see Section 4.4.2). Under such circumstances, one could imagine a scenario in which the facultative benefit comes about by the provisioning of an additional amount of some factor. If the host ceased to produce this factor on its own, this would turn the facultative mutualism into an obligate symbiosis. It is unclear, however, how likely such shifts are in nature.

Lastly, facultative or obligate mutualisms might evolve into stable ultimate mutualisms (transition 6 in Figure 4.2). In particular, evolved dependence is considered a possible precursor to ultimate mutualism because it couples the evolutionary fates of host and symbiont. Subsequent selection could then fine-tune the interaction and act on *Wolbachia* to confer some 'extra' benefit (Aanen and Hoekstra, 2007). Furthermore, mutual dependence should lead to co-speciation between host and symbiont. However, although host dependence on *Wolbachia* has evolved frequently (see Section 4.4), co-speciation between *Wolbachia* and arthropod hosts has never been found among mutualistic strains (and only rarely among parasitic strains; Raychoudhury *et al.*, 2009). This might suggest that obligate mutualisms between *Wolbachia* and arthropods are not stable on an evolutionary timescale or at least too short-lived to evolve into ultimate mutualisms. Obligate mutualisms might become unstable if the host is able to overcome the dependence (transition 7 in Figure 4.2); in addition, these relationships could in general be more prone to extinction (Kremer *et al.*, 2009a). On the whole, the fact that mutualisms between *Wolbachia* and arthropod hosts appear to be quite dynamic, even on ecological timescales, makes it hard to predict the evolutionary fate of such associations.

4.7 Future directions

The study of *Wolbachia* mutualisms in arthropods is a young field of research, and several issues await further investigation. Here, we point to some promising avenues for future research.

How are *Wolbachia*-induced mutualisms achieved mechanistically?

It will be of great importance to elucidate in more detail the mechanisms that underlie mutualistic effects. So far, some insights have been gained regarding obligate mutualisms (e.g. *Wolbachia*'s role in progressing *A. tabida* egg chambers past the mid-oogenesis checkpoint by preventing apoptosis of nurse cells). Still, the mechanisms underlying other cases of evolved dependence remain unclear. Furthermore, the molecular nature of most mutualisms is unknown, particularly that of facultative benefits. In light of common mechanisms involved in mutualistic and parasitic phenotypes, unravelling the mechanistic basis of *Wolbachia* mutualisms might also help to better understand how these symbionts manipulate host reproduction.

Is host protection only a temporary phenomenon?

Wolbachia frequently triggers immune responses in newly infected hosts, but does so rarely in hosts adapted to infection. This suggests that protection associated with immune upregulation might only be a transient effect. On the other hand, *Wolbachia*-induced protection is not necessarily associated with immune activation. It is therefore crucial to elucidate the mechanism(s) underlying anti-pathogenic effects of natural *Wolbachia* infections, particularly the exact role of the host immune system. In Chapter 6, we review the current knowledge of the interactions between *Wolbachia* and the insect immune system and conclude that anti-pathogenic effects are likely to diminish with ongoing symbiont-host coevolution. A better understanding of the physiological causes of anti-pathogenic effects will help to characterize such effects as largely ephemeral or as effective benefits of *Wolbachia* infection.

How stable are mutualistic interactions between *Wolbachia* and arthropods?

Not only protective effects, but *Wolbachia*–arthropod mutualisms in general should be tested for their stability. This issue revolves around the question of how likely transitions between different forms of symbiosis are (Figure 4.2). In other words, how frequently do mutualisms arise, and how fast are they lost? Our understanding of this matter with respect to *Wolbachia*–arthropod relationships is still very limited. In contrast to the situation in *Wolbachia*-infected nematodes, co-speciation between mutualistic *Wolbachia* and arthropods has not yet been found, indicating that these mutualisms are relatively short-lived and might easily break down in evolutionary (or

even in ecological) time. On the other hand, mutualisms might just be difficult to detect at all. These questions require further investigation.

Can we identify ultimate benefits provided by *Wolbachia*?

The search for *Wolbachia*-induced ultimate mutualisms is still in its infancy. Ultimate mutualisms relate to interactions in which a partner could never have performed as well without the other (as opposed to evolved dependencies). In order to detect ultimate benefits, it is necessary to compare the performance of two different host genotypes, one being infected with and adapted to *Wolbachia* and the other one being uninfected and adapted to the symbiont's absence (De Mazancourt *et al.*, 2005). Future studies should try to apply this method to identify ultimate benefits (although they are difficult to measure).

Are insects more prone to *Wolbachia* mutualisms than other arthropods?

It is striking that almost all cases of mutualistic *Wolbachia*–arthropod relationships have been found among insect species. This may be because non-insect arthropods have only rarely been tested for *Wolbachia* mutualisms, or because mutualisms have evolved less frequently in these host species. To discern between these possibilities, future work should intensify the search for mutualistic *Wolbachia* effects in non-insect host species such as spiders, isopods, and mites.

Did *Wolbachia* mutualisms foster the evolution of haplodiploidy?

Although not discussed in this review, *Wolbachia* mutualisms could also be relevant to the question of whether male-killing endosymbionts possibly play a role in the evolution of haplodiploidy in their hosts. Recent theory suggests that slight benefits accruing from infection facilitate the evolution of haplodiploidy, whereas earlier models that do not consider possible mutualisms often fail to explain the evolution of haplodiploidy by endosymbionts (Kuijper and Pen, 2010). Researchers could look for empirical support for these theoretical findings.

4.8 Conclusions

In addition to their notorious reproductive parasitism, *Wolbachia* also have the potential to engage in mutualistic relationships with their hosts.

As mutualists, *Wolbachia* either provide facultative fitness benefits or are required for host survival or reproduction (obligate mutualism).

Not only can *Wolbachia* be mutualistic, they also frequently act as a mutualist and as a reproductive parasite at the same time ('Jekyll and Hyde' type of infection). Moreover, they can induce both mutualism and reproductive parasitism by interfering with the same host process (e.g. iron metabolism). These findings argue against a clear-cut distinction between parasitic and mutualistic *Wolbachia* and imply that transitions between both forms of symbiosis might occur relatively easily.

Facultative mutualisms arise through selection on maternally transmitted *Wolbachia* to enhance the fitness of their female hosts. Such fitness benefits have been found in different arthropod species and include increases in fecundity and longevity, nutritional provisioning, and protection against pathogens. Obligate mutualisms arise through the evolution of dependence, either via compensatory evolution in the host (tolerance) or via the takeover of some host function by *Wolbachia*. Tolerance has evolved frequently in arthropod hosts as a means to cope with the harmful effects of *Wolbachia* infection. Since tolerance strategies tend to render hosts dependent on *Wolbachia*, they are a potential barrier to the evolution of host resistance (as are direct fitness benefits, too).

In contrast to the abundance of experimental studies that found *Wolbachia*-induced pathogen interference (anti-pathogenic effects), there is only limited support for a fitness-enhancing effect of such interference in natural interactions (i.e. for host protection). Many studies observed anti-pathogenic effects after *Wolbachia* had been artificially introduced into naturally uninfected insects. While these findings may offer great potential for disease control strategies, they say little about natural *Wolbachia*–host interactions. Moreover, there is broad evidence that *Wolbachia*-associated protection has not evolved for the purpose of protecting the host, but rather is a side effect of other processes and hence an instance of byproduct benefits. Lastly, protection that is based on a host immune response might only be a temporary phenomenon.

Once *Wolbachia* are established in a host population, providing a fitness benefit is not necessary for them to be maintained, because reproductive parasitism is sufficient for this purpose. However, reproductive parasitism alone is often insufficient for *Wolbachia* to invade a population (for example because the bacteria fail to overcome the invasion threshold). By contrast, facultative mutualisms enable *Wolbachia* to establish and spread from low initial frequencies and therefore facilitate the invasion into novel hosts (this holds even if beneficial effects are only temporary).

Both facultative and obligate *Wolbachia* mutualisms have further important consequences for the ecology, evolution, and development of their arthropod hosts. Effects can be as diverse as the requirement of bacterial signalling for oogenesis, manipulation of host plant physiology (e.g. induction of green islands on yellow leaves), or irreversible loss of sexual reproduction. The mechanisms that underlie *Wolbachia* mutualisms are likewise diverse (as far as they are known), including alterations in gene expression and interference with crucial host processes such as apoptosis and sex determination.

5 Evolution of *Wolbachia* with direct fitness benefits

It is increasingly acknowledged that *Wolbachia* can also have beneficial effects on host fitness, either along with reproductive parasitism ('Jekyll and Hyde' infections) or not ('stand-alone benefit' infections). In this chapter, we analyze the effect of direct fitness benefits on the evolution of *Wolbachia*, using the examples of cytoplasmic incompatibility and male killing. By means of a simple population genetic model, we derive invasion conditions and equilibrium frequencies for the different infection scenarios. Our results demonstrate the importance of a strain's 'effective fitness' (i.e. the product of bacterial transmission efficiency and relative fitness of an infected female) for its invasion success. In the case of 'Jekyll and Hyde' infections, direct fitness benefits substantially facilitate their invasion and spread, for example by lowering or removing the invasion threshold. Moreover, for *Wolbachia* strains with weak or no reproductive parasitism, fitness benefits make invasion possible in the first place. Finally, we discuss the role of direct fitness benefits in long-term evolutionary dynamics of reproductive phenotypes and highlight their potential to resolve genetic conflicts between maternally inherited symbionts and their hosts.

A slightly different version of this chapter has been published in *Heredity* (Zug and Hammerstein, 2018).

5.1 Introduction

In light of the drastic phenotypes of reproductive parasitism, it is not surprising that there has been a historical tendency to separate symbionts into mutualists and reproductive parasites. This view, however, neglects the fact that both strategies are not mutually exclusive: as we have seen in Chapter 4, *Wolbachia* can also have direct beneficial effects on host fitness, in addition to fitness effects that are due to reproductive parasitism. Instances of reproductive parasitism with direct fitness benefits have been termed 'Jekyll and Hyde' infections (Jiggins and Hurst, 2011). Traditionally, models describing the infection dynamics of *Wolbachia* either ignore direct fitness effects altogether or consider only fitness costs of infection. For example, MK models usually lack a term describing direct fitness effects, and most CI models consider direct fitness effects only in terms of reduced fecundity of infected females (but see Randerson *et al.*, 2000; Dobson *et al.*, 2002). Only recently have researchers begun to include the possibility of direct fitness benefits when modeling the invasion and spread of *Wolbachia*. However, such fitness benefits have mostly been modeled in the form of symbiont-mediated protection against pathogens (Fenton *et al.*, 2011; Souto-Maior *et al.*, 2015). Such models usually involve many specific assumptions and parameters concerning pathogen infection, e.g. virulence, susceptibility, or parasitism rate. Hence, these models are not applicable to cases where symbiont-induced fitness benefits do not stem from protective effects. Moreover, the significance of *Wolbachia*-mediated protection in nature is still under debate (see Chapter 4). Therefore, a more general conception of direct fitness benefits is needed when modeling the infection dynamics of 'Jekyll and Hyde' infections. Last but not least, direct fitness benefits are also crucial for understanding the prevalence of infections where reproductive parasitism is weak or absent ('stand-alone benefit' infections; see Chapter 4). Theory suggests that, in these cases, and if transmission is not perfect, symbionts should increase host fitness in order to be maintained (Hoffmann and Turelli, 1997; Kriesner *et al.*, 2013, 2016).

In this chapter, we analyze the effects of direct fitness benefits on the dynamics of both 'stand-alone benefit' and 'Jekyll and Hyde' infections. In the latter case, we focus on CI and MK, the two most commonly observed reproductive phenotypes. We investigate infection dynamics both with one and two *Wolbachia* strains and also consider the possibility of doubly infected host individuals. We derive invasion conditions and equilibrium frequencies for the different infection scenarios. Finally, we discuss our findings in the light of relevant theoretical and empirical research.

5.2 Model

In our population genetic model, we assume a single panmictic host population with discrete, non-overlapping generations. We further assume haploid individuals that reproduce sexually with the primary sex ratio being 1:1. Individuals are sufficiently described by two parameters: their infection status and their sex. Regarding their infection status, individuals can be uninfected, infected with CI-inducing *Wolbachia*, with MK-*Wolbachia*, or with both (only in Section 5.3.3). Moreover, we include the possibility of direct fitness benefits associated with infection, either along with reproductive parasitism ('Jekyll and Hyde' infections) or not ('stand-alone benefit' infections). *Wolbachia* are maternally inherited with transmission efficiency t, i.e. the proportion of infected offspring produced by an infected female (for all parameters used in this model, see Table 5.1). 'Jekyll and Hyde' infections are assumed to be transmitted as efficient as their counterparts without direct fitness benefits. Regarding double infections, transmission of CI is assumed to be independent of transmission of MK.

Depending on infection status and sex, individuals can be grouped into different classes. We denote by p the frequency of females of a given class, as a fraction of the whole population (for reasons of comprehensibility, however, frequencies depicted in the figures are those of all individuals of a given class, i.e. males and females). We derive difference equations describing the frequency changes of the different classes of individuals from one generation to the next. Building upon standard replicator dynamics, frequencies of the offspring classes can be calculated from frequencies of parental classes and fitness-related effects of infection. *Wolbachia*-induced fitness effects can be partitioned into two components: those due to reproductive parasitism (CI and/or MK in this chapter), and those directly affecting host fitness. Both fitness effects are assumed to be independent of each other.

5.2.1 Fitness effects due to reproductive parasitism

Under CI, uninfected females suffer offspring loss when mating with infected males. This fitness reduction is depicted as the CI level l_{CI}, i.e. the proportion of offspring killed in incompatible matings. Under MK, male offspring is killed during embryogenesis. However, a fraction v of the male offspring is assumed to survive the male-killing. A proportion β of the resources the killed males would have used is reallocated to the surviving siblings and distributed equally among them. The relative fitness of the surviving

Table 5.1: Glossary of notation.

Symbol	Definition
β	resource reallocation efficiency (proportion of the resources the killed males would have used that is reallocated to the surviving siblings)
F	relative fitness of infected females
l_{CI}	CI level (proportion of offspring killed in incompatible matings)
p	infection frequency (proportion of infected females)
$\hat{p}$	equilibrium infection frequency
$p_{\oplus}$	beneficial infection frequency
p_{CI}	CI infection frequency
p_{MK}	MK infection frequency
$p_{\mathrm{CI+MK}}$	CI+MK double infection frequency
p_{U}	frequency of uninfected females
p^{ini}	initial infection frequency
p^{thr}	threshold infection frequency
R	fitness compensation (surviving offspring's fitness increase through resource reallocation)
t	transmission efficiency (proportion of infected offspring produced by an infected female)
v	male viability in the face of MK

offspring from infected females is increased by a factor R which is given by

$$R = 1 + \frac{\beta t_{\mathrm{MK}}(1-v)}{2 - t_{\mathrm{MK}}(1-v)}. \tag{5.1}$$

For the derivation of this fitness compensation term, see for example Normark (2004) and Engelstädter and Hurst (2006a).

5.2.2 Direct fitness effects

We denote by the parameter F the relative fitness of an infected female. In the classical CI models by Hoffmann and Turelli (Hoffmann *et al.*, 1990; Turelli, 1994; Hoffmann and Turelli, 1997), F denotes the relative fecundity of infected females, but it is not necessary to confine fitness to fecundity. In fact, the first theoretical analysis of CI uses a broad definition by considering a general 'selective advantage' of uninfected females (Caspari and Watson, 1959). Here we adopt this broad definition and

let F comprise any direct effect of infection on female fitness, including fecundity, survival, growth, and performance (note that fitness effects due to reproductive manipulations are not measured by F). In doubly infected individuals, direct fitness effects of each *Wolbachia* strain are assumed to be independent of each other.

A general conception of direct fitness effects has two major advantages: First, by including any possible fitness-related trait, the model also captures symbiont-induced fitness effects that are unrelated to fecundity, e.g. longevity (Fry and Rand, 2002), development time (Xue *et al.*, 2012), and larval competitiveness (Gavotte *et al.*, 2010). This is particularly relevant because laboratory measurements of fecundity are highly sensitive to assay conditions, in contrast to other fitness traits measured (Ackermann *et al.*, 2001). Second, our model is independent of the particular mechanism underlying the fitness effect, e.g. anti-pathogenic protection (Zélé *et al.*, 2012), nutritional provisioning (Brownlie *et al.*, 2009), or manipulation of host plant physiology (Kaiser *et al.*, 2010).

Turelli (1994), who used F as relative fecundity, named the term Ft (i.e. the product of relative fecundity and bacterial transmission efficiency of an infected female) the 'effective fecundity' of the strain infecting her. Based on our terminology, we accordingly refer to Ft as the 'effective fitness' of a symbiont and thus broaden the scope of this approach. In this chapter, we show that effective fitness is crucial for determining a symbiont's invasion potential.

Analytical calculations were performed by hand and using Mathematica 9.0 (Wolfram Research, Inc.). Programs were written, and simulations were performed using Matlab 8.1 (The MathWorks, Inc.).

5.3 Results

5.3.1 Infection dynamics with one strain

'Stand-alone benefit' infection dynamics

In the absence of reproductive manipulations, *Wolbachia* must confer a fitness benefit to the host in order to be able to spread ('stand-alone benefit' infections). In describing the infection dynamics of facultative beneficial symbionts, at least two parameters are necessary: the transmission efficiency $t_{\oplus}$, and the relative fitness of infected females $F_{\oplus}$. The proportion p of infected and uninfected females in the population then changes from one

generation to the next by

$$p'_{\oplus} = \frac{p_{\oplus} F_{\oplus} t_{\oplus}}{\overline{w}}, \tag{5.2a}$$

$$p'_{\mathrm{U}} = \frac{p_{\oplus} F_{\oplus} (1 - t_{\oplus}) + p_{\mathrm{U}}}{\overline{w}}, \tag{5.2b}$$

where

$$\overline{w} = 2 \left(p_{\oplus} F_{\oplus} + p_{\mathrm{U}} \right).$$

The resulting stable equilibrium frequency of beneficial *Wolbachia* strains is

$$\hat{p}_{\oplus} = \frac{F_{\oplus} t_{\oplus} - 1}{2 \left(F_{\oplus} - 1 \right)}. \tag{5.3}$$

This equilibrium frequency is precisely analogous to mutation-selection balance for haploids (Hoffmann and Turelli, 1997). The 'transmission-selection balance' described by Equation (5.3) is the simplest means by which a polymorphism for a beneficial infection can be maintained (Jaenike, 2012).

The condition for the spread of beneficial symbionts is

$$F_{\oplus} t_{\oplus} > 1. \tag{5.4}$$

We see that for beneficial *Wolbachia* to spread, their effective fitness must be larger than one. If, on the other hand, $F_{\oplus} t_{\oplus} < 1$, symbionts will not spread by benefits alone. They then have to resort to some kind of reproductive manipulation such as CI or MK in order to persist.

CI infection dynamics

In describing the infection dynamics of CI-inducing *Wolbachia*, we follow earlier models (Hoffmann *et al.*, 1990; Turelli, 1994; Hoffmann and Turelli, 1997). The proportion p of infected and uninfected females in the population changes from one generation to the next by

$$p'_{\mathrm{CI}} = \frac{(p_{\mathrm{CI}} F_{\mathrm{CI}} t_{\mathrm{CI}}) \left[p_{\mathrm{CI}} + p_{\mathrm{U}} \right]}{\overline{w}}, \tag{5.5a}$$

$$p'_{\mathrm{U}} = \frac{(p_{\mathrm{CI}} F_{\mathrm{CI}} (1 - t_{\mathrm{CI}}) + p_{\mathrm{U}}) \left[p_{\mathrm{CI}} (1 - l_{\mathrm{CI}}) + p_{\mathrm{U}} \right]}{\overline{w}}, \tag{5.5b}$$

where

$$\overline{w} = 2\left(p_{\mathrm{U}}\left(p_{\mathrm{CI}}\left(1-l_{\mathrm{CI}}\right)+p_{\mathrm{U}}\right)+p_{\mathrm{CI}}F_{\mathrm{CI}}\left(p_{\mathrm{CI}}\left(1-l_{\mathrm{CI}}\left(1-t_{\mathrm{CI}}\right)\right)+p_{\mathrm{U}}\right)\right).$$

In the numerator of Equations (5.5a) and (5.5b), the first term (in round brackets) denotes the maternal contribution, and the second term (in square brackets) denotes the paternal contribution. Note that, under CI, the proportion of infected and uninfected individuals among males equals that among females, hence it is possible to express the paternal contribution in terms of female frequencies.

Equation (5.5a) has two nontrivial equilibria, given by

$$\hat{p}_{\mathrm{CI}} = \frac{1-F_{\mathrm{CI}}+l_{\mathrm{CI}}+\sqrt{\left(F_{\mathrm{CI}}-l_{\mathrm{CI}}-1\right)^2-A\left(1-F_{\mathrm{CI}}t_{\mathrm{CI}}\right)}}{A}, \quad (5.6a)$$

$$p_{\mathrm{CI}}^{\mathrm{thr}} = \frac{1-F_{\mathrm{CI}}+l_{\mathrm{CI}}-\sqrt{\left(F_{\mathrm{CI}}-l_{\mathrm{CI}}-1\right)^2-A\left(1-F_{\mathrm{CI}}t_{\mathrm{CI}}\right)}}{A}, \quad (5.6b)$$

where

$$A = 4l_{\mathrm{CI}}\left(1-F_{\mathrm{CI}}+F_{\mathrm{CI}}t_{\mathrm{CI}}\right).$$

The first equilibrium is stable and defines the CI equilibrium frequency. The second equilibrium is unstable and defines the threshold frequency below which the infection will disappear from the population. The CI threshold exists whenever $F_{\mathrm{CI}}t_{\mathrm{CI}} < 1$ and can take values of substantial magnitude, particularly under low transmission efficiency and high costs of infection, i.e. reduced fitness (Figure 5.1, top). By contrast, if a strain's effective fitness is larger than one ($F_{\mathrm{CI}}t_{\mathrm{CI}} > 1$), there is no invasion threshold (Figure 5.1, bottom). Since $t_{\mathrm{CI}} \leq 1$, this is only possible if $F_{\mathrm{CI}} > 1$. Hence, if a CI strain confers a small fitness benefit so that $F_{\mathrm{CI}}t_{\mathrm{CI}} > 1$, it can invade and spread from any initial frequency, however small it may be, and will eventually reach the equilibrium frequency $\hat{p}_{\mathrm{CI}}$ given by Equation (5.6a) (Figure 5.2, top).

It has been stated that changes in F_{CI} have little impact on $\hat{p}_{\mathrm{CI}}$ (Weeks *et al.*, 2007). However, this is only correct as long as the initial frequency $p_{\mathrm{CI}}^{\mathrm{ini}}$ exceeds the threshold frequency $p_{\mathrm{CI}}^{\mathrm{thr}}$, a situation that is rather unrealistic. If, more realistically, the initial frequency lies below the threshold frequency, changes in F_{CI} have a huge effect on $\hat{p}_{\mathrm{CI}}$ (Figure 5.2, bottom). For a large part of the range of the CI level l_{CI}, the relationship between F_{CI} and $\hat{p}_{\mathrm{CI}}$ exhibits a switch-like behavior at the point where the initial frequency equals the threshold frequency. Above this point, the positive effect of F_{CI}

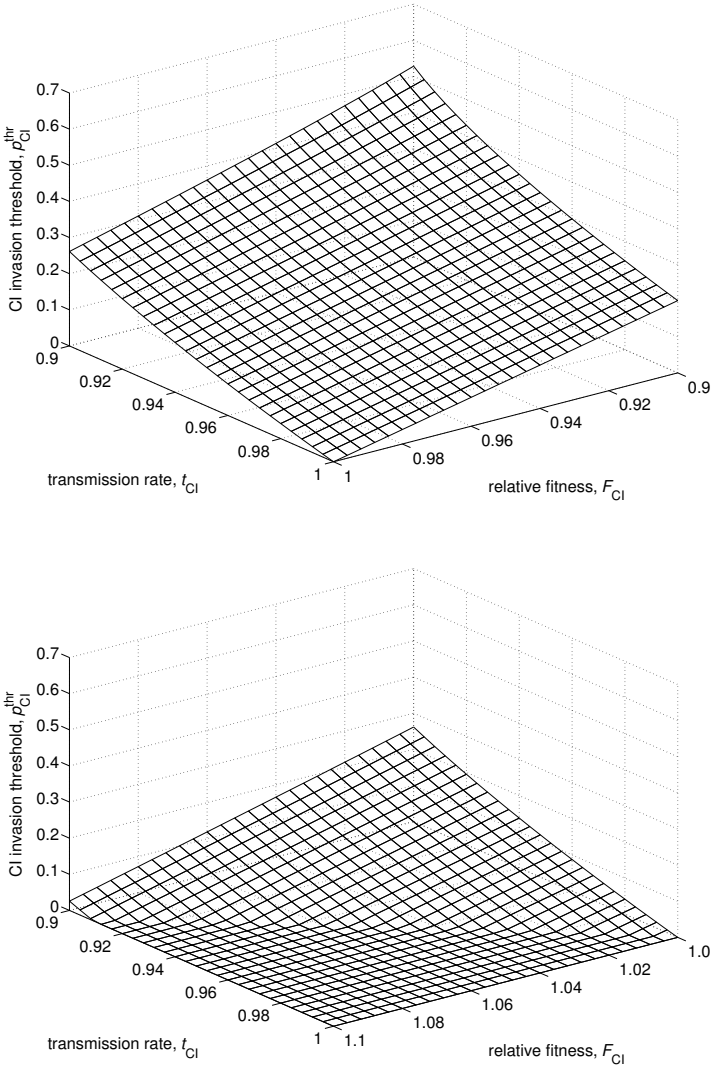
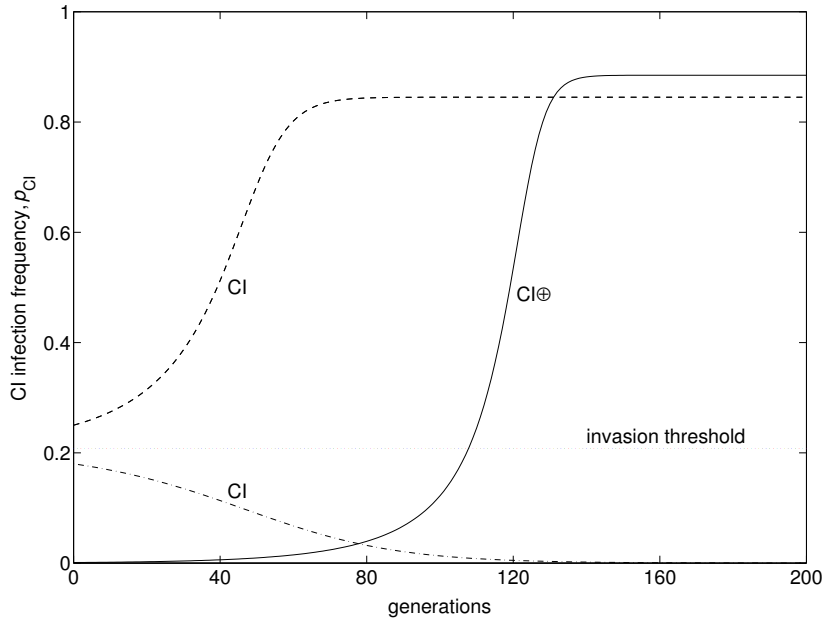


Figure 5.1: Direct fitness benefits facilitate invasion of CI by lowering or removing the CI invasion threshold. Behavior of the CI invasion threshold $p_{\mathrm{CI}}^{\mathrm{thr}}$ in dependence of the relative fitness of infected females, F_{CI}, and the transmission rate, t_{CI}, for $F_{\mathrm{CI}} < 1$ (top) and $F_{\mathrm{CI}} > 1$ (bottom). The CI threshold disappears if $F_{\mathrm{CI}} t_{\mathrm{CI}} > 1$. $l_{\mathrm{CI}} = 0.5$.


CI infection frequency, p_{CI}
generations
CI
CI⊕
CI
invasion threshold
0
0.2
0.4
0.6
0.8
1
0
40
80
120
160
200

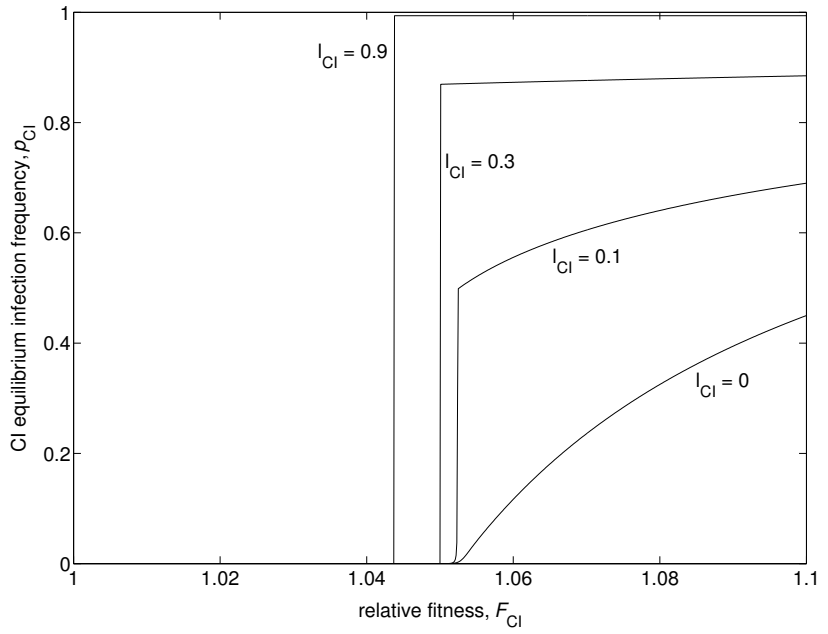

CI equilibrium infection frequency, p_{CI}
relative fitness, F_{CI}
I_{CI} = 0.9
I_{CI} = 0.3
I_{CI} = 0.1
I_{CI} = 0
0
0.2
0.4
0.6
0.8
1
1
1.02
1.04
1.06
1.08
1.1

Figure 5.2 (previous page): Direct fitness benefits have a strong effect on CI equilibrium frequency. Top: Dynamics of CI infection frequency p_{CI} with and without direct fitness benefits. If the symbiont induces CI only, its initial frequency must exceed a given threshold (thin dotted line) in order to spread (dashed line); otherwise it will be lost from the population (dash-dotted line). If it additionally confers a fitness benefit so that $F_{\mathrm{CI}}t_{\mathrm{CI}} > 1$ (CI$\oplus$), it will spread from any initial frequency (solid line). Bottom: The effect of fitness benefits on CI equilibrium frequency $\hat{p}_{\mathrm{CI}}$ for different l_{CI} values, given a low initial frequency. A small fitness benefit is unable to raise the infection above the threshold. For most l_{CI} values, a switch-like increase in $\hat{p}_{\mathrm{CI}}$ occurs where $p_{\mathrm{CI}}^{\mathrm{ini}} = p_{\mathrm{CI}}^{\mathrm{thr}}$. Above this point, the positive effect of F_{CI} is small for larger l_{CI} values, but large for small l_{CI} values. Parameters take the values $F_{\mathrm{CI}} = 1$, $F_{\mathrm{CI}\oplus} = 1.1$, $l_{\mathrm{CI}} = 0.3$ (top), and $t_{\mathrm{CI}} = 0.95$.

on $\hat{p}_{\mathrm{CI}}$ is extremely small for a large part of the l_{CI} range, but becomes strong for small l_{CI} values. The switch-like behavior only vanishes for very small l_{CI} values close to zero, where $\hat{p}_{\mathrm{CI}}$ increases gradually with increasing F_{CI}.

MK infection dynamics

In describing the infection dynamics of MK-inducing *Wolbachia*, we follow earlier models, in particular Hurst (1991b), Randerson *et al.* (2000), and Normark (2004). The proportion p of infected and uninfected females in the population changes from one generation to the next by

$$p'_{\mathrm{MK}} = \frac{p_{\mathrm{MK}} R F_{\mathrm{MK}} t_{\mathrm{MK}}}{\overline{w}}, \tag{5.7a}$$

$$p'_{\mathrm{U}} = \frac{p_{\mathrm{U}} + p_{\mathrm{MK}} R F_{\mathrm{MK}} (1 - t_{\mathrm{MK}})}{\overline{w}}, \tag{5.7b}$$

where

$$\overline{w} = p_{\mathrm{MK}} \left(R F_{\mathrm{MK}} t_{\mathrm{MK}} (1 + v) + 2 R F_{\mathrm{MK}} (1 - t_{\mathrm{MK}}) \right) + 2 p_{\mathrm{U}}.$$

In Equations (5.7a) and (5.7b), the numerator denotes only the maternal contribution (as the paternal contribution cancels out in both equations).

The nontrivial equilibria of these equations are

$$\hat{p}_{\mathrm{MK}} = \frac{RF_{\mathrm{MK}}t_{\mathrm{MK}} - 1}{B}. \tag{5.8a}$$

$$\hat{p}_{\mathrm{U}} = \frac{RF_{\mathrm{MK}}\left(1 - t_{\mathrm{MK}}\right)}{B}, \tag{5.8b}$$

where

$$B = RF_{\mathrm{MK}}\left(2 - t_{\mathrm{MK}}\left(1 - v\right)\right) - \left(1 + v\right).$$

In contrast to CI, MK distorts offspring sex ratios, and therefore the proportion of infected females differs from that of infected males (that are scarce or absent under MK). To derive the equilibrium frequency of infected males, one has to multiply $\hat{p}_{\mathrm{MK}}$ with v.

The MK equilibrium $\hat{p}_{\mathrm{MK}}$ is positive if

$$RF_{\mathrm{MK}}t_{\mathrm{MK}} > 1. \tag{5.9}$$

Since this is also the condition for $\hat{p}_{\mathrm{MK}}$ to be stable (not shown), Condition (5.9) is a necessary and sufficient condition for male-killing bacteria to invade and persist in a population. Hence, male killers can invade if the product of their effective fitness ($F_{\mathrm{MK}}t_{\mathrm{MK}}$) and the fitness compensation through resource reallocation (R) is larger than one.

It is convenient to solve this invasion condition for β, the resource reallocation efficiency (Hurst, 1991b; Normark, 2004). Doing this yields

$$\beta > \frac{(1 - F_{\mathrm{MK}}t_{\mathrm{MK}})(2 - t_{\mathrm{MK}}(1 - v))}{F_{\mathrm{MK}}t_{\mathrm{MK}}^2(1 - v)}. \tag{5.10}$$

If the resource reallocation efficiency β is greater than a critical value β^{crit} (given by Condition (5.10)), then the male killer will invade and finally reach the equilibrium frequency given in Equation (5.8a). If MK-bacteria have a negative fitness effect on infected females ($F_{\mathrm{MK}} < 1$), then β^{crit} can take quite high values, particularly if there is considerable male survival under MK (Figure 5.3, top). The ability of male killers to induce a fitness benefit ($F_{\mathrm{MK}} > 1$) substantially facilitates their invasion by lowering the critical resource reallocation efficiency β^{crit} (Figure 5.3, bottom). Therefore, even if the resource reallocation efficiency β is very low, a small fitness benefit is sufficient to reduce the critical value β^{crit} so that the male killer can invade. The critical value β^{crit} exists whenever $F_{\mathrm{MK}}t_{\mathrm{MK}} < 1$. That is the same condition as for the existence of the CI invasion threshold (Equation (5.6b)).

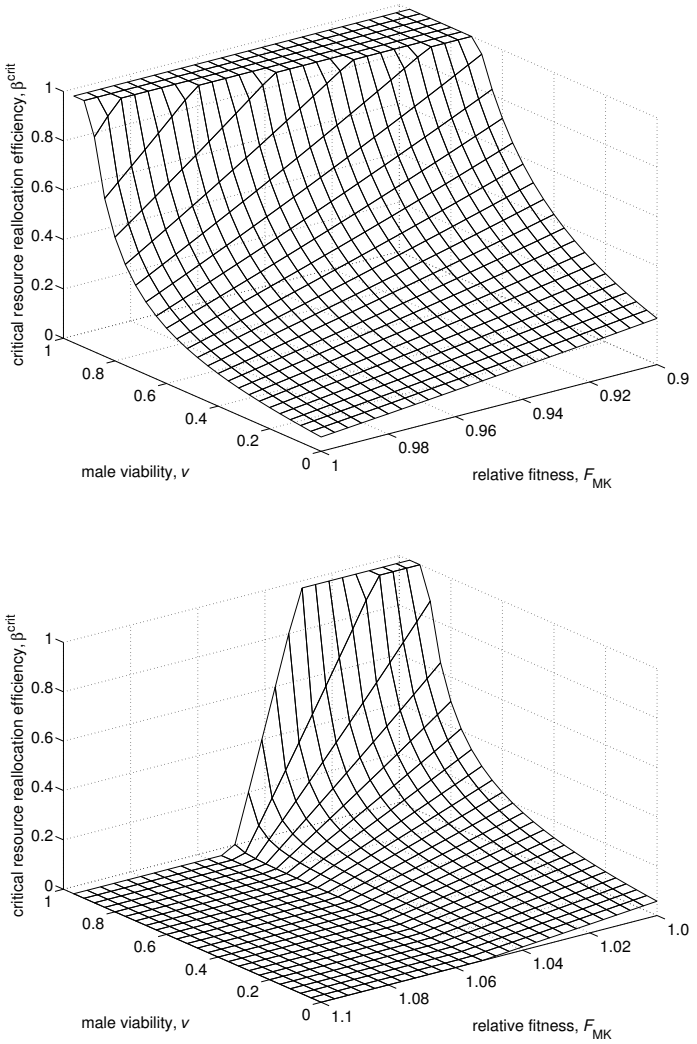


Figure 5.3: Direct fitness benefits facilitate invasion of male killers by lowering or removing the critical resource reallocation efficiency. Behavior of the critical resource reallocation efficiency, β^{crit}, in dependence of the relative fitness of infected females, F_{MK}, and the viability of males under MK, v, for$F_{\mathrm{MK}} < 1$ (top) and $F_{\mathrm{MK}} > 1$ (bottom). β^{crit} disappears if $F_{\mathrm{MK}} t_{\mathrm{MK}} > 1$. $t_{\mathrm{MK}} = 0.95$.

This again emphasizes the importance of a symbiont's effective fitness for its invasion potential.

In general, a fitness benefit has a positive effect on the MK equilibrium frequency $\hat{p}_{\mathrm{MK}}$, but the strength of the effect depends on other parameters such as β and v. The positive effect of F_{MK} on $\hat{p}_{\mathrm{MK}}$ is weak for large values of β and small values of v, but strong for small values of β and large values of v (not shown).

5.3.2 Infection dynamics with two strains, but without doubly infected hosts (coinfection at the population level)

For the rest of this chapter (infection dynamics with two strains), we present in the main text the invasion conditions and their biological implications. For the difference equations and the derivation of the invasion conditions, we refer the reader to the Appendix.

Two strains: beneficial vs. CI

Invasion of a beneficial strain into a CI population. As shown in Appendix 1, the condition for an initially rare beneficial strain to increase in a population where a CI strain is at equilibrium is

$$F_{\oplus}t_{\oplus}(1 - 2l_{\mathrm{CI}}\hat{p}_{\mathrm{CI}}) > F_{\mathrm{CI}}t_{\mathrm{CI}}. \tag{5.11}$$

$F_{\oplus}t_{\oplus}$ is the effective fitness of the beneficial strain, and $F_{\mathrm{CI}}t_{\mathrm{CI}}$ is the effective fitness of the CI strain. The term $(1 - 2l_{\mathrm{CI}}\hat{p}_{\mathrm{CI}})$ describes the CI-associated offspring loss in females not infected with the CI strain. Thus, in order to invade, a beneficial strain must not only exhibit a larger effective fitness than the resident CI strain, but also compensate for the fitness loss due to incompatible matings with CI-infected males (Turelli, 1994). The latter is only possible with low CI levels. If condition (5.11) is fulfilled, the beneficial strain will invade and drive the CI strain to extinction. For biologically reasonable parameter values ($F_{\mathrm{CI}}, t_{\mathrm{CI}} > 0.9$), the term $F_{\mathrm{CI}}t_{\mathrm{CI}}/(1 - 2l_{\mathrm{CI}}\hat{p}_{\mathrm{CI}})$ is always larger than one (not shown). Hence, if Condition (5.11) is satisfied, it also implies that $F_{\oplus}t_{\oplus} > 1$; therefore, the beneficial strain will finally reach the equilibrium frequency $\hat{p}_{\oplus}$ given by Equation (5.3).

If the beneficial strain is able to rescue CI (i.e. if it is a $mod^{-}resc^{+}$ strain), the invasion condition is simply

$$F_{\oplus}t_{\oplus} > F_{\mathrm{CI}}t_{\mathrm{CI}}. \tag{5.12}$$

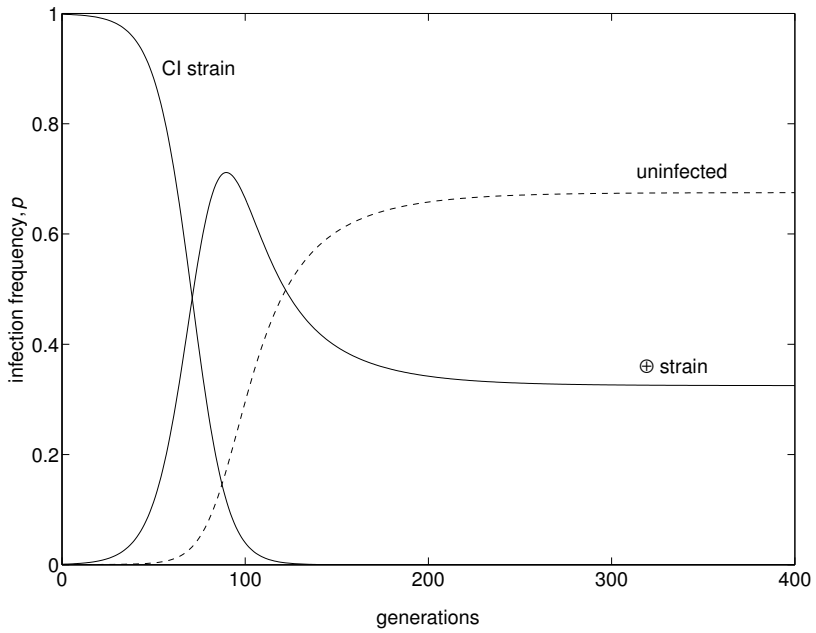


Figure 5.4: A beneficial symbiont that is able to rescue CI can invade even into populations fixed for CI. Such invasion is possible if Condition (5.12) is fulfilled. Parameters take the values $F_{\oplus} = 1.08$, $t_{\oplus} = 0.95$, $F_{\mathrm{CI}} = 0.95$, $t_{\mathrm{CI}} = 0.98$, and $l_{\mathrm{CI}} = 0.98$.

Whether the ability to rescue CI influences the invasion probability of a beneficial strain depends on which of the Conditions (5.11) and (5.12) are fulfilled. If both are satisfied, the beneficial strain will invade and reach $\hat{p}_{\oplus}$, regardless of whether it is able to rescue CI or not. Importantly, however, insensitivity to CI significantly accelerates the time it takes for the beneficial strain to invade the population and replace the CI strain (not shown). If neither condition is satisfied, infection will go extinct in both cases. Finally, if Condition (5.12) is satisfied, but Condition (5.11) is not, it is crucial whether the beneficial strain is able to rescue CI or not. If not (i.e. if it is $mod^{-}resc^{-}$), it will go extinct. However, if the beneficial strain is $mod^{-}resc^{+}$, it will invade the population, drive the resident CI strain to extinction and, if $F_{\oplus}t_{\oplus} > 1$, finally reach the equilibrium frequency $\hat{p}_{\oplus}$. The fact that neither l_{CI} nor $\hat{p}_{\mathrm{CI}}$ enters Condition (5.12) implies that a $mod^{-}resc^{+}$ beneficial strain is able to invade a CI population even if CI

levels are very high and CI is essentially fixed in the population (Figure 5.4).

Theory suggests that, with imperfect maternal transmission and without any fitness benefits, $mod^{-}resc^{+}$ strains can only spread in the presence of a $mod^{+}resc^{+}$ strain, but their spread eventually leads to the extinction of both infections (Hurst and McVean, 1996). Beneficial $mod^{-}resc^{+}$ strains, by contrast, can easily spread in a CI-infected population and outcompete the resident strain.

Invasion of a CI strain into a beneficial population. The condition for a rare CI strain to invade a population in which a beneficial infection is at equilibrium was derived by Kriesner *et al.* (2013) and is given by

$$F_{\mathrm{CI}} t_{\mathrm{CI}} > F_{\oplus} t_{\oplus}. \tag{5.13}$$

Because essentially no CI occurs when the CI strain is very rare, the incompatibility does not enter this condition. Hence, for a CI strain to invade a population at equilibrium with a beneficial strain it is sufficient that it has a larger effective fitness (obviously, this also holds for a beneficial strain invading a population infected with another beneficial strain). However, a beneficial strain can reach its equilibrium frequency in the first place only if $F_{\oplus} t_{\oplus} > 1$. Without direct fitness benefits, the CI strain's effective fitness cannot exceed 1, and it is therefore only able to invade a population infected at equilibrium with a beneficial strain if it confers a direct fitness benefit.

Two strains: beneficial vs. MK

Invasion of a beneficial strain into a MK population. The condition for an initially rare beneficial strain to increase in a population where a MK strain is at equilibrium is

$$F_{\oplus} t_{\oplus} > R F_{\mathrm{MK}} t_{\mathrm{MK}}. \tag{5.14}$$

Thus, in order to invade, a beneficial strain's effective fitness must exceed the MK strain's effective fitness multiplied by the fitness compensation factor R.

Invasion of a MK strain into a beneficial population. The condition for an initially rare MK strain to invade into a population infected at equilibrium with a beneficial strain is

$$R F_{\mathrm{MK}} t_{\mathrm{MK}} > F_{\oplus} t_{\oplus}. \tag{5.15}$$

Above we showed that a CI strain without direct fitness benefits is not able to invade a beneficial population (section 5.3.2). By contrast, a MK strain without direct benefits is able to do so if the fitness compensation due to MK is sufficiently high.

Two strains: CI vs. MK

Invasion of a MK strain into a CI population. The condition for an initially rare MK strain to increase in a population where a CI strain is at equilibrium is

$$RF_{\mathrm{MK}}t_{\mathrm{MK}}(1-2l_{\mathrm{CI}}\hat{p}_{\mathrm{CI}}) > F_{\mathrm{CI}}t_{\mathrm{CI}}. \tag{5.16}$$

The invading MK strain faces the risk of high offspring mortality due to CI prevailing in the population. Therefore, with high CI levels, invasion is not possible for reasonable parameter values. In addition to low CI levels, invasion is also facilitated by high fitness compensation through male-killing (R) and direct fitness benefits of the MK strain. A strong male killer (with low v and high β values, leading to high fitness compensation) might be able to invade into a CI population even without conferring direct fitness benefits. However, as shown in section 5.3.1, fitness benefits strongly enhance the invasion potential of weak male killers (high v and low β). In particular, if a male killer's resource reallocation efficiency β is low, the symbiont will only be able to invade when conferring a direct fitness benefit.

If the MK strain is $mod^{-}resc^{+}$, the invasion condition simplifies to

$$RF_{\mathrm{MK}}t_{\mathrm{MK}} > F_{\mathrm{CI}}t_{\mathrm{CI}}. \tag{5.17}$$

If Condition (5.17) is fulfilled, the $mod^{-}resc^{+}$ MK strain will invade the population, drive the resident CI strain to extinction, and, if $RF_{\mathrm{MK}}t_{\mathrm{MK}} > 1$, finally reach the equilibrium frequency $\hat{p}_{\mathrm{MK}}$. Given that the $mod^{-}resc^{+}$ MK strain is insensitive to CI, it can invade even in populations fixed for CI.

So far, we have considered fitness compensation only in the case of male-killing. However, fitness compensation could also occur in broods where offspring is killed not because of MK, but CI (Freeland and McCabe, 1997). Interestingly, when vertical transmission is high (which holds true throughout this chapter), such compensation cannot aid the spread of CI, but only increases the average fitness of uninfected offspring, and may thus even impede the spread of CI (Freeland and McCabe, 1997). In our model, we also find this impeding effect of CI-associated fitness compensation: when

we allow for fitness compensation not only for MK, but also for CI, we find that invasion of a MK strain into a CI population is accelerated significantly (not shown). Therefore, the possibility of CI-associated fitness compensation does not prevent CI strains from being replaced by MK strains (given that Condition (5.16) or (5.17) is fulfilled).

Invasion of a CI strain into a MK population. Due to frequency-dependent selection, CI involves a minimum infection frequency below which it cannot establish. When introduced into a population infected with male killers, this invasion threshold exists whenever $F_{\mathrm{CI}}t_{\mathrm{CI}} < RF_{\mathrm{MK}}t_{\mathrm{MK}}$ (albeit we could not derive this threshold analytically). If, on the other hand, $F_{\mathrm{CI}}t_{\mathrm{CI}} > RF_{\mathrm{MK}}t_{\mathrm{MK}}$, there is no invasion threshold, and CI can establish no matter how low its initial frequency. Therefore, the condition for an initially arbitrarily rare CI strain to increase in a population where a MK strain is at equilibrium is

$$F_{\mathrm{CI}}t_{\mathrm{CI}} > RF_{\mathrm{MK}}t_{\mathrm{MK}}. \tag{5.18}$$

The fact that the invasion condition does not contain the CI level l_{CI} implies that even strains with very low CI levels can invade. This is not surprising, however, since a strain with stand-alone benefits is also able to invade a MK population as long as its effective fitness is large enough (see above). Further, remember that the condition for male killers to invade an uninfected population is $RF_{\mathrm{MK}}t_{\mathrm{MK}} > 1$ (Condition (5.9)). Therefore, for a male killer to reach the equilibrium, the right-hand side of Condition (5.18) must be larger than one. From this, it is easy to see that an extremely rare CI strain without direct benefits cannot invade a population infected at equilibrium with a male killer, because its effective fitness never exceeds one. Hence, invasion is only possible for a CI strain that additionally confers a direct fitness benefit (the same holds true for CI invasion into a population infected at equilibrium with a beneficial symbiont; see above).

5.3.3 Infection dynamics with two strains and doubly infected hosts (coinfection at the individual level)

So far, we have not observed stable coexistence of CI and MK. Previous modeling has shown that stable coexistence of CI- and MK-*Wolbachia* is possible if doubly infected individuals exist within the population (Engelstädter *et al.*, 2004). If doubly infected hosts occur, there are eight types of individuals, four of each sex. The corresponding equation system

(see Appendix A2) has eight equilibrium sets, five of which are biologically plausible. These plausible sets are (i) the infection-free equilibrium, (ii) the CI equilibrium (stable), (iii) the CI threshold (unstable), (iv) the MK equilibrium, and (v) the equilibrium containing CI, MK, and the double infection CI+MK. In the latter case, all eight types of individuals are present in equilibrium, and the equilibrium frequencies of the four female types are given by

$$\hat{p}_{\mathrm{CI+MK}} = \frac{\left(1 - F_{\mathrm{CI}} + l_{\mathrm{CI}} + \sqrt{C}\right)\left(RF_{\mathrm{MK}}t_{\mathrm{MK}} - 1\right)}{D}, \tag{5.19a}$$

$$\hat{p}_{\mathrm{CI}} = \frac{\left(1 - F_{\mathrm{CI}} + l_{\mathrm{CI}} + \sqrt{C}\right) RF_{\mathrm{MK}}\left(1 - t_{\mathrm{MK}}\right)}{D}, \tag{5.19b}$$

$$\begin{aligned}\hat{p}_{\mathrm{MK}} = &-\left(1 - F_{\mathrm{CI}}\left(1 - 2l_{\mathrm{CI}}\left(1 - t_{\mathrm{CI}}\right)\right) - l_{\mathrm{CI}} + \sqrt{C}\right)\\ &\times\left(RF_{\mathrm{MK}}t_{\mathrm{MK}} - 1\right)\frac{1}{D},\end{aligned} \tag{5.19c}$$

$$\begin{aligned}\hat{p}_{\mathrm{U}} = &-\left(1 - F_{\mathrm{CI}}\left(1 - 2l_{\mathrm{CI}}\left(1 - t_{\mathrm{CI}}\right)\right) - l_{\mathrm{CI}} + \sqrt{C}\right)\\ &\times RF_{\mathrm{MK}}\left(1 - t_{\mathrm{MK}}\right)\frac{1}{D},\end{aligned} \tag{5.19d}$$

where

$$\begin{aligned}C &= \left(F_{\mathrm{CI}} - l_{\mathrm{CI}} - 1\right)^2 - 4l_{\mathrm{CI}}\left(1 - F_{\mathrm{CI}}t_{\mathrm{CI}}\right)\left(1 - F_{\mathrm{CI}} + F_{\mathrm{CI}}t_{\mathrm{CI}}\right),\\ D &= 2l_{\mathrm{CI}}\left(1 - F_{\mathrm{CI}} + F_{\mathrm{CI}}t_{\mathrm{CI}}\right)\left(RF_{\mathrm{MK}}\left(2 - t_{\mathrm{MK}}\left(1 - v\right)\right) - \left(1 + v\right)\right).\end{aligned}$$

Note that the term inside the square root, C, is identical with the term inside the square root in the standard CI equilibria (Equations (5.6a) and (5.6b)), and the denominator, D, is half the product of the denominators in the standard CI and MK equilibria (Equations (5.6a) and (5.8a)). Accordingly, the equilibrium frequency of the double infection $\hat{p}_{\mathrm{CI+MK}}$ (Equation (5.19a)) is twice the product of the equilibrium frequencies of the corresponding single infections $\hat{p}_{\mathrm{CI}}$ (Equation (5.6a)) and $\hat{p}_{\mathrm{MK}}$ (Equation (5.8a)) in the one-strain dynamics. Moreover, there is a structural similarity between $\hat{p}_{\mathrm{CI+MK}}$ and $\hat{p}_{\mathrm{CI}}$ on the one hand, and between $\hat{p}_{\mathrm{MK}}$ and $\hat{p}_{\mathrm{U}}$ on the other hand. One reason for this is that both uninfected females and females infected by a male killer suffer from CI, whereas females infected by CI+MK and CI do not. Hence, if all four types are present in equilibrium, we can expect generally larger values for $\hat{p}_{\mathrm{CI+MK}}$ and $\hat{p}_{\mathrm{CI}}$ than for $\hat{p}_{\mathrm{MK}}$ and $\hat{p}_{\mathrm{U}}$ (Figure 5.5, bottom).

Table 5.2: Possible outcomes of the introduction of the double infection.

	$C_1 > 1$?	$C_2 > 1$?	$C_3 > 1$?	Further conditions	Result (stable equilibria)
I	no	no	no		U
II	no	no	yes		MK, U
III	yes	no	yes	CI and MK parameters high[1]	CI+MK, CI, MK, U[2]
				else	MK, U
IV	no	yes	no		CI, U
V	yes	yes	no		CI, U
VI	yes	yes	yes		CI+MK, CI, MK, U[2]

[1] Here, CI parameters are F_{CI}, l_{CI}, t_{CI}, and MK parameters are F_{MK}, t_{MK}, β.
[2] For most of the parameter range, CI+MK and CI dominate over MK and U (see main text).

Invasion of a CI+MK double infection into an uninfected population

If we allow for the existence of doubly infected hosts, complex dynamics are possible. The condition for an initially rare double infection (CI+MK) to invade into an uninfected population and finally reach the stable equilibrium given by Equation (5.19a) is

$$F_{\mathrm{CI}} t_{\mathrm{CI}} R F_{\mathrm{MK}} t_{\mathrm{MK}} > 1. \tag{5.20}$$

If this condition is not met, the double infection must exceed a threshold frequency in order to be able to invade (Engelstädter *et al.*, 2004).

To see how Condition (5.20) relates to the possible outcomes of the CI+MK introduction, let us term its left-hand part C_1 and partition it into two parts, C_2 and C_3:

$$\overbrace{\underbrace{F_{\mathrm{CI}} t_{\mathrm{CI}}}_{C_2} \underbrace{R F_{\mathrm{MK}} t_{\mathrm{MK}}}_{C_3}}^{C_1}$$

Table 5.2 shows all possible outcomes of the introduction of a double infection into an uninfected population, dependent on which of the terms C_1, C_2 and C_3 are larger than one. Let us start with the obvious results. If none of the three conditions is fulfilled, there will be no infection (case I;

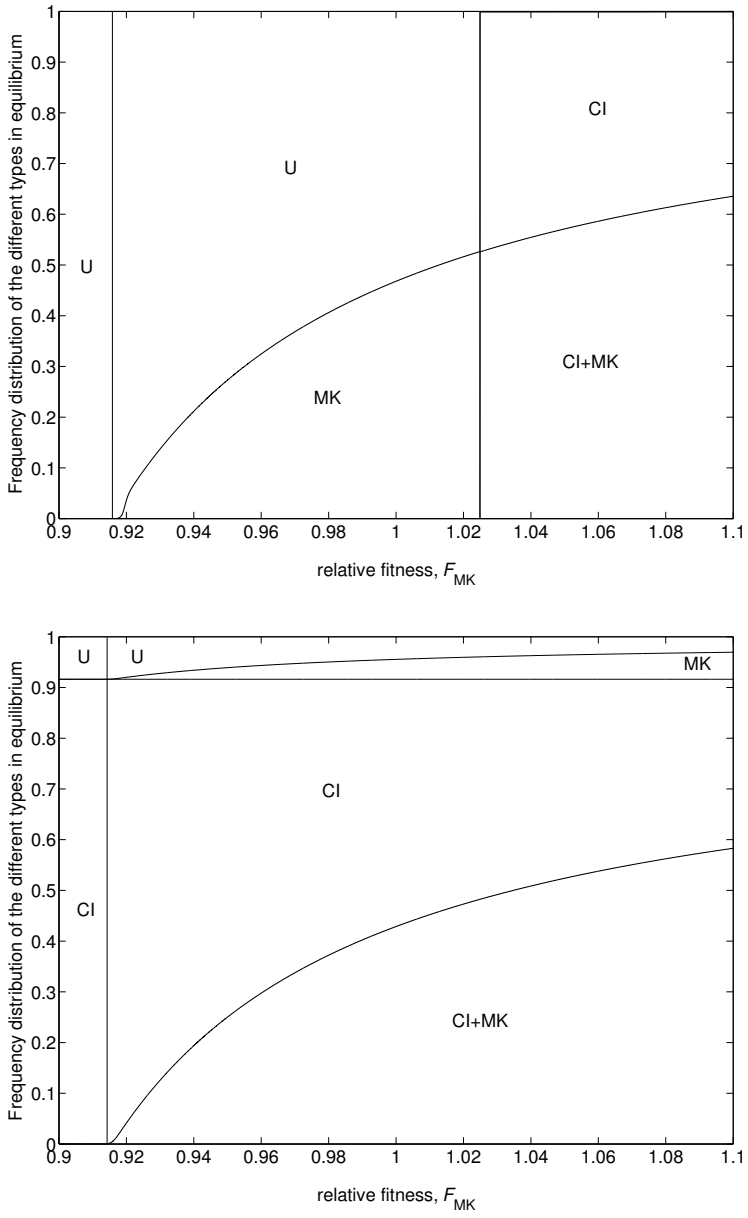


Figure 5.5: Frequency distribution of the different types in equilibrium after invasion of a CI+MK double infection into an uninfected population. Frequency distributions are shown in dependence of the relative fitness of females infected with the male-killer, F_{MK}, for $F_{\mathrm{CI}} < 1$ (top) and $F_{\mathrm{CI}} > 1$ (bottom). Parameters take the values $F_{\mathrm{CI}} = 0.96$, $l_{\mathrm{CI}} = 0.9$ (top), $F_{\mathrm{CI}} = 1.02$, $l_{\mathrm{CI}} = 0.1$ (bottom), $t_{\mathrm{CI}} = 0.99$, $t_{\mathrm{MK}} = 0.95$, $\beta = 0.2$, and $v = 0.1$.

Figure 5.5, top). If only $C_3 > 1$, only the male killer will invade (case II; Figure 5.5, top), and if only $C_2 > 1$, only CI will establish (case IV; Figure 5.5, bottom). The double infection is stably maintained only if $C_1 > 1$, that is, if Condition (5.20) is fulfilled (cases III and VI; Figure 5.5). Importantly, however, this condition is necessary, but not sufficient for the stable existence of double infections. Even if Condition (5.20) is fulfilled, it is possible that the double infection disappears, while either CI or MK prevails as a single infection (cases III and V).

The most interesting dynamics can be observed in case III. Here, the fact that $RF_{\mathrm{MK}}t_{\mathrm{MK}} > 1$ implies that MK will be present in equilibrium, be it as single or double infection. In contrast, the effective fitness of the CI strain is smaller than one. In the one-strain dynamics, this implies the existence of a threshold frequency so that no CI invasion is possible if the initial frequency is below this threshold (see section 5.3.1). The introduction of a double infection, by contrast, makes it possible for CI to establish in a population, even if extremely rare initially. To see this, let us have a closer look at the infection dynamics in case III. Due to imperfect maternal transmission ($t_{\mathrm{CI}}, t_{\mathrm{MK}} < 1$), the double infection also introduces both single infections into the population. Given that the CI-associated drive is frequency-dependent (being very weak when CI is rare), whereas the MK-associated drive is not, MK will initially increase more strongly than CI. Nevertheless, as long as the double infection increases, the single CI infection will also increase. With increasing CI+MK and CI frequencies, CI-associated positive frequency-dependent selection becomes stronger, so that it can happen that CI eliminates MK and becomes predominant, being present both as single and double infection. This happens if sufficient parameters underlying the success of CI and MK (F_{CI}, l_{CI}, t_{CI}, F_{MK}, t_{MK}, β) are large enough (while male viability v must not be too large). The crucial point is that CI benefits from a successful double infection, regardless of how this success is achieved. This leads to the counter-intuitive result that even an increase in F_{MK} can result in the displacement of MK by the double infection and in fixation of CI (Figure 5.5, top).

Invasion of a CI+MK double infection into a CI population

The condition for an initially rare double infection (CI+MK) to increase in a population where CI is at equilibrium is

$$RF_{\mathrm{MK}}t_{\mathrm{MK}} > 1. \tag{5.21}$$

With the spread of the double infection, the single MK infection is also introduced into the population. The only exception is for $l_{\mathrm{CI}} = 1$: in a population fixed for CI, the double infection can invade, but there is no introduction of the single MK infection. Instead, CI stays fixed during the invasion process; before the invasion, the single CI infection was fixed, and after the invasion, there is a polymorphism of the single CI and the double infection.

If Condition (5.21) is not met, the double infection will not be able to invade, however high its initial frequency may be. The reason is that it is only MK that must establish (CI is already at equilibrium), but MK is not frequency-dependent so that there is no extra benefit from a high initial frequency.

Invasion of a CI+MK double infection into a MK population

The condition for an initially rare double infection (CI+MK) to increase in a population where MK is at equilibrium is

$$F_{\mathrm{CI}} t_{\mathrm{CI}} > 1. \tag{5.22}$$

With the spread of the double infection, the single CI infection is also introduced into the population. There is a tendency for the CI+MK infection to 'replace' the MK infection, eventually reaching an equilibrium frequency very similar to the initial MK frequency, and equally for the CI infection to 'replace' the uninfecteds. This tendency increases with increasing l_{CI}, until replacement is perfect with complete CI (Figure 5.6). That is because, for $l_{\mathrm{CI}} = 1$, $\hat{p}_{\mathrm{CI+MK}}$ (Equation (5.19a)) = $\hat{p}_{\mathrm{MK}}$ (Equation (5.8a)), and $\hat{p}_{\mathrm{CI}}$ (Equation (5.19b)) = $\hat{p}_{\mathrm{U}}$ (Equation (5.8b)). As a consequence, and not surprisingly for $l_{\mathrm{CI}} = 1$, CI becomes fixed in the population, being present as a single and a double infection.

If Condition (5.22) is not met, the double infection must again exceed a threshold frequency in order to be able to invade (Engelstädter *et al.*, 2004).

5.4 Discussion

Reproductive parasitism constitutes a powerful means for maternally inherited symbionts to spread in host populations. However, evidence is accumulating that, in a range of circumstances, reproductive manipulation alone is not sufficient to ensure *Wolbachia* invasion and spread. In these

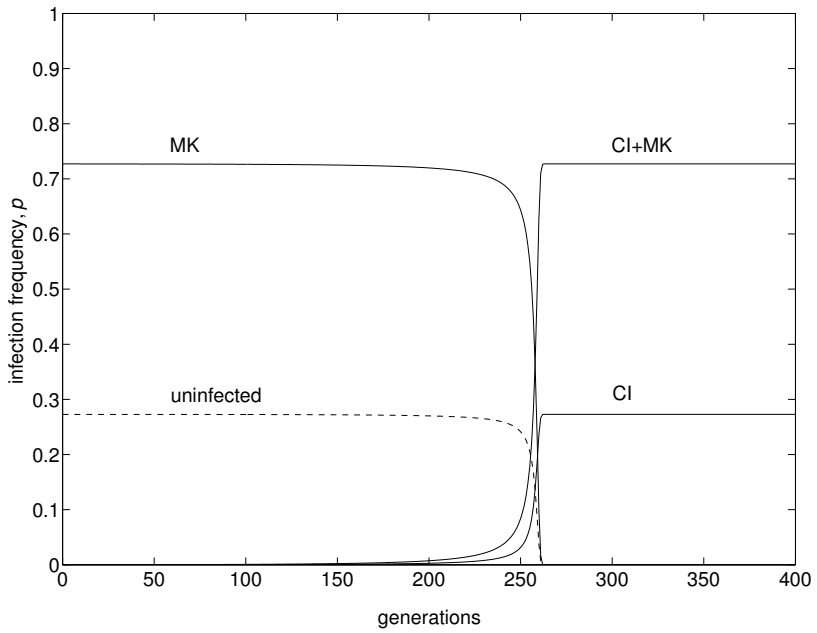


Figure 5.6: With complete CI, the invading double infection completely replaces the pre-existing MK infection. Simultaneously, CI is introduced as a single infection. As a consequence, CI becomes fixed in the population. Parameters take the values $F_{\mathrm{CI}} = 1.04$, $t_{\mathrm{CI}} = 0.98$, $l_{\mathrm{CI}} = 1$, $F_{\mathrm{MK}} = 1.05$, $t_{\mathrm{MK}} = 0.97$, $\beta = 0.2$, and $v = 0.1$.

cases, direct positive effects on female fitness are predicted to enable symbionts to successfully invade host populations. This study investigates the effects of direct fitness benefits on the evolution of reproductive parasites, using the examples of CI and MK.

The key quantity in our model is effective fitness, i.e., the product of the relative fitness of an infected female and her transmission efficiency. This parameter thus captures any direct effect of infection on female fitness (but no fitness effects that are due to reproductive parasitism). Ever since Turelli (1994) established the concept of effective fitness, theoretical studies have repeatedly proven its significance for the evolutionary success of microbes inducing CI or MK (Randerson *et al.*, 2000; Egas *et al.*, 2002; Vavre *et al.*, 2003; Vautrin *et al.*, 2007, 2008; Haygood and Turelli, 2009; Kriesner *et al.*, 2016). Our study supports these findings and at the

same time extends them in several ways. First, most previous studies only consider the narrow version of effective fitness, that is, effective fecundity. Our broad approach thus widens the applicability of the concept. Second, for the first time, we apply the concept of effective fitness to a situation where different types of reproductive parasites co-occur, thus unifying earlier results. Lastly, we actually include positive values for a strain's effective fitness. Despite strong evidence of direct fitness benefits, this has been largely ignored in previous models.

From our model, several major conclusions can be drawn: Frequently, direct fitness benefits significantly facilitate invasion, e.g. by lowering or even removing the invasion threshold. In other cases, direct benefits make invasion possible in the first place. Furthermore, taking into account positive fitness effects allows for a more general view of the dynamics of multiple infections with different reproductive manipulations. Here we discuss these findings and their implications in more detail.

One of the major gaps in our understanding of the infection dynamics of reproductive parasites is how they initially invade host populations. When introduced into a novel host, *Wolbachia* frequently perform poorly, for example exhibiting low transmission efficiency (Clancy and Hoffmann, 1997; Heath *et al.*, 1999; Rigaud *et al.*, 2001; Riegler *et al.*, 2004). Moreover, the benefits of reproductive parasitism are not unconditional. The drive associated with CI is frequency-dependent and hence very weak when infection is rare. As a result, CI-inducing *Wolbachia* face a threshold frequency below which infection cannot establish. Therefore, recently introduced CI infections, which usually exhibit low frequencies, should fail to invade (although, in principle, chance fluctuations might carry low initial frequencies above the threshold; Jansen *et al.* 2008). The drive associated with MK does not depend on infection frequency, but on the efficacy of the MK-associated fitness compensation, measured in terms of male-killing intensity and resource reallocation efficiency. This equally results in a threshold behavior: if fitness compensation is not effective enough, the male killer will fail to invade. Indeed, survival of males despite infection with MK-*Wolbachia* has been observed (Hurst *et al.*, 2000; Dyer and Jaenike, 2004; Charlat *et al.*, 2005; Weinert *et al.*, 2007). In addition, it is unclear how efficient resource reallocation is in nature (Balas *et al.*, 1996; Martins *et al.*, 2010; Elnagdy *et al.*, 2011). Accordingly, there has also been some debate in the theoretical literature on the range of values for the resource reallocation efficiency (Engelstädter and Hurst, 2006a; Úbeda and Normark, 2006). In sum, invading *Wolbachia* face a range of obstacles when relying solely on reproductive parasitism. Our results

show that direct fitness benefits easily overcome these obstacles by lowering or even removing invasion thresholds, thus confirming previous theoretical findings in the specific context of symbiont-induced protection (Fenton *et al.*, 2011; Souto-Maior *et al.*, 2015). Such net fitness advantages seem far more plausible than chance fluctuations to pass the invasion threshold (Turelli and Barton, 2017). Lastly, reproductive parasitism may be weak or absent. Our findings confirm that in such cases, direct fitness benefits enable *Wolbachia* to invade host populations in the first place. Again, this is in line with recent theoretical results (Kriesner *et al.*, 2013, 2016), and a recent empirical study corroborates that *Wolbachia* that do not show reproductive parasitism in a novel host rapidly evolve to be benign (Veneti *et al.*, 2012).

Direct fitness benefits also influence infection dynamics of different types of reproductive parasites within one host population. Engelstädter *et al.* (2004) showed that, without direct benefits, threshold frequencies for CI-*Wolbachia* are highest if they are introduced as a single infection into a population infected with MK-*Wolbachia* at equilibrium, and that the opposite invasion (MK-symbionts into CI-infected population) is not possible under strong CI. Our results show that, in the former case, direct benefits easily remove the invasion threshold, and that, in the latter case, direct benefits considerably facilitate invasion of a male killer, in particular if it is a weak one (low β, high v). Moreover, a $mod^{-}resc^{+}$ male killer can invade even in populations fixed for CI. Evidence of the existence of such a CI-insensitive MK strain comes from *Drosophila pandora* that carries both CI- and MK-*Wolbachia*. No incompatibility was detected in crosses between CI-infected males and MK-infected females, suggesting that these females can rescue CI (Richardson *et al.*, 2016). Unfortunately, there is as yet not much empirical evidence for invasion scenarios involving CI and MK within one host population. The tropical butterfly *Hypolimnas bolina* might be a promising system to study the interactions between both reproductive phenotypes. In the South Pacific, this species harbours two strains of *Wolbachia*, one of which induces MK and the other one induces CI. Most populations are infected with either the MK strain or the CI strain only, suggesting that, most often, populations infected by one strain are able to resist invasion by the other strain, and vice versa (Charlat *et al.*, 2006). These findings are in line with the theoretical prediction that CI and MK cannot stably coexist in a single host population (if no doubly infected individuals occur; Engelstädter *et al.*, 2004). In 3 out of 25 populations, however, co-occurrence of both strains was observed. The most likely explanation is that these populations are not at equilibrium, but

rather represent transitional stages in which one strain (in this case, the CI strain) is about to invade and replace the other (Charlat *et al.*, 2006). Given the existence of a threshold frequency for CI-*Wolbachia* introduced into a population infected with a male-killer, it is quite possible that the invasion of CI into MK-infected *H. bolina* populations is facilitated by direct fitness benefits.

Theory suggests that CI- and MK-*Wolbachia* can coexist within a single unstructured host population in two different ways. First, both phenotypes can be expressed simultaneously by the same strain (Hurst *et al.*, 2002). Alternatively, CI and MK are expressed by different *Wolbachia* strains, and doubly infected host individuals exist (Freeland and McCabe, 1997; Engelstädter *et al.*, 2004). The latter situation was investigated in this chapter. To the best of our knowledge, we present for the first time invasion conditions and equilibrium frequencies for the invasion of CI+MK double infections. Engelstädter *et al.* (2004) showed via simulations that the double infection must exceed a threshold frequency to invade a population that is uninfected or MK-infected, but that this threshold is lower than the threshold for CI as a single infection. In accordance with these results, we find that invasion conditions for the double infection are more relaxed than those for the single infections. Our results show that direct fitness benefits also facilitate invasion of double infections by reducing or removing the threshold. Evidence for the role of direct benefits in the invasion of double infections is scarce. Although CI- and MK-*Wolbachia* co-occur in *Drosophila pandora* (Richardson *et al.*, 2016), we cannot yet assess the role of direct fitness benefits in the persistence of CI+MK double infections in *D. pandora* populations.

Lastly, we may take a look at how direct fitness benefits affect evolutionary dynamics of *Wolbachia* in the long run. In particular, evolutionary dynamics of CI have been the focus of extensive research. Early models showed that the *mod* function is selectively neutral, predicting that a $mod^{-}resc^{+}$ strain would spread if it raised effective fitness (Prout, 1994; Turelli, 1994). Although these studies did not consider host population structure, which has been argued to engender selection for stronger CI through a kin selection process (Hurst, 1991a; Frank, 1997), a more recent model found that, even in subdivided host populations, selection for increased CI levels is only weak and transient (Haygood and Turelli, 2009). These findings have two major implications. First, regardless of whether host populations are panmictic or structured, selection on CI-inducing *Wolbachia* acts to maximize effective fitness, and not the CI level itself. This has also been shown to hold for haplodiploid hosts (Egas *et al.*, 2002; Vavre *et al.*, 2003)

and multiple infections (Vautrin *et al.*, 2007, 2008). The fact that there is selection acting on effective fitness, but not on CI intensity also implies that CI and direct fitness benefits are not correlated. Both predictions have been confirmed for CI-inducing *Wolbachia* in *Drosophila simulans*: In Californian fly populations, *Wolbachia* have evolved so that effective fitness, but not CI intensity, has risen (Weeks *et al.*, 2007). Moreover, recent experiments revealed that, across multiple *Wolbachia* strains, CI and antiviral protection occur independently (Martinez *et al.*, 2015). These observations are inconsistent with the view that CI is a pleiotropic byproduct of other bacterial traits that benefit hosts, as has been assumed by Prout (1994) and Turelli (1994). The second major implication concerns the possible extinction of CI-inducing *Wolbachia*. Given the lack of selection for the *mod* function, symbionts may lose their ability to induce CI and become extinct (Hurst and McVean, 1996). Moreover, theory predicts that host resistance to CI will be selected for in infected males and uninfected females (Rousset *et al.*, 1991; Turelli, 1994; Koehncke *et al.*, 2009), and there is some evidence in line with that prediction (Reynolds and Hoffmann, 2002; Tortosa *et al.*, 2010; Raychoudhury and Werren, 2012). The evolution of host suppressor genes may eventually also lead to extinction of CI-inducing *Wolbachia*. However, loss of infection is not expected if symbionts additionally have a beneficial effect on host fitness (which is quite likely, given selection for higher effective fitness). In the presence of direct fitness benefits, symbionts may still lose the ability to induce CI, but probably won't go extinct. In this scenario, *Wolbachia* that used to express CI subsequently lost this ability (either because of lacking positive selection or because the host evolved suppression) and now are maintained solely by beneficial effects.

In the case of MK, evolutionary pressures are somewhat different from those acting on CI-inducing *Wolbachia*. For male killers, the primary target of selection is the product of effective fitness and MK-associated fitness compensation. This was first shown by Randerson *et al.* (2000) who called the quantity the 'Basic Rate of Increase' (BRI). Hence, and in contrast to CI, male killers are under selection to increase not only effective fitness, but also the efficiency of the reproductive phenotype itself. Nevertheless, male killers also face the risk of extinction, and that is because of strong selection on hosts to counteract the sex ratio distortion (Werren, 1987; Hurst, 1992; Randerson *et al.*, 2000). Host suppression of the action or transmission of MK-*Wolbachia* has been observed in several *Drosophila* species (Jaenike, 2007), in the dwarf spider *Oedothorax gibbosus* (Vanthournout and Hendrickx, 2016), and in different populations of the

butterfly *Hypolimnas bolina* (Hornett *et al.*, 2006; Charlat *et al.*, 2007; Mitsuhashi *et al.*, 2011). The spread of MK suppression can be very rapid, shifting an extremely female-biased population sex ratio to an even one in under 10 generations, which suggests intense selection for suppression (Charlat *et al.*, 2007). Interestingly, however, suppression of the MK phenotype in *H. bolina* does not lead to reduced frequency or even extinction of *Wolbachia* (Charlat *et al.*, 2005; Hornett *et al.*, 2006). That is because the bacteria immediately express CI when MK is suppressed, which is sufficient to maintain the infection in the population (Hornett *et al.*, 2008). Another possible reason for the maintenance of infection is that *Wolbachia* confer a direct fitness benefit to the host. In this case, the bacteria are predicted to persist as well, even if the MK phenotype is suppressed. Direct fitness benefits therefore could explain the persistence of male-killing *Wolbachia* in populations where suppression of MK has presumably evolved (as deduced from the occurrence of infected males; e.g. Weinert *et al.*, 2007). Eventually, populations once plagued by MK would end up harbouring only "beneficial symbionts that are a peaceful resolution of an evolutionary arms race between a male-killer and a suppressor system" (Majerus and Majerus, 2010).

In this spirit, one can speculate on the evolutionary fate of the fitness benefits themselves. It is well known that mutualistic interactions are highly context-dependent (Bronstein, 1994; Chamberlain *et al.*, 2014). Accordingly, benefits provided by *Wolbachia* are likely to depend on the environmental conditions experienced by the host (for example, presence or absence of a natural enemy against which the symbiont provides protection; see Chapter 4). Therefore, interactions between beneficial symbionts and their hosts are supposed to be highly dynamic (Jaenike, 2012), involving frequent evolutionary transitions in the outcome of symbiosis and blurring the line between mutualism and parasitism (Sachs *et al.*, 2011a,b). Taken together, these considerations suggest that both reproductive manipulations and direct fitness benefits might appear and disappear quickly on an evolutionary timescale.

In summary, our results demonstrate that direct fitness benefits have a strong effect on the invasion and persistence of *Wolbachia*. Positive fitness effects enable or facilitate invasion into novel hosts and allow symbionts to persist even in the absence of reproductive manipulations. Beneficial effects also play an important role in infection dynamics involving more than one manipulation phenotype (both at the population and the individual level), but more empirical research is clearly needed in this respect. Our findings also point to the potential of direct fitness benefits to resolve genetic conflicts

between maternally inherited symbionts and their hosts. As more and more beneficial aspects of *Wolbachia* and other reproductive manipulators are being discovered, their significance for the evolution of these symbionts and their hosts is increasingly acknowledged. It will be exciting to further elucidate the interplay of reproductive manipulations and mutualistic effects in ensuring the evolutionary success of CI- and MK-inducing *Wolbachia*.

6 *Wolbachia* and the arthropod immune system

The mechanisms underlying *Wolbachia* phenotypes, both parasitic and mutualistic, are only poorly understood. Moreover, it is unclear how the arthropod immune system is involved in these phenotypes and why it is not more successful in eliminating the bacteria. In this chapter, we argue that reactive oxygen species (ROS) are likely to be key in elucidating these issues. ROS are major players in the arthropod immune system, and *Wolbachia* infection can affect ROS levels in the host. After reviewing the essentials of arthropod immunity, we elaborate a hypothesis that considers the different effects of *Wolbachia* on the immune system of novel and native hosts, with a focus on the oxidative environment. We propose that newly introduced *Wolbachia* trigger an immune response and cause oxidative stress, whereas in coevolved symbioses, infection is not associated with oxidative stress, but rather with restored redox homeostasis. Redox homeostasis can be restored in different ways, depending on whether *Wolbachia* or the host is in charge. This hypothesis offers a mechanistic explanation for several of the observed *Wolbachia* phenotypes.

A slightly different version of this chapter has been published in *Frontiers in Microbiology* (Zug and Hammerstein, 2015b).

6.1 Introduction

Reactive oxygen species (ROS) have long been viewed as purely harmful molecules contributing to oxidative stress, which can cause severe cell damage. On the other hand, ROS can also play a beneficial role, for example in intracellular signaling and innate immune defense. *Wolbachia* have recently been shown to influence ROS production and the oxidative environment as a whole, suggesting an involvement of ROS in *Wolbachia*-induced phenotypes. In this chapter, we first give an overview of the arthropod immune response with a focus on ROS. In the main part, we outline the interactions between *Wolbachia* and the host immune system. We explore the possible roles of ROS in different *Wolbachia* phenotypes and hypothesize how interference with the host oxidative environment has shaped various aspects of the *Wolbachia*–arthropod symbiosis. Finally, we discuss some corollaries of the hypothesis.

6.2 Reactive oxygen species, oxidative stress, and redox homeostasis

In eukaryotic cells, aerobic respiration takes place in the mitochondria. Electrons are transferred along the mitochondrial respiratory chain to generate a proton gradient which eventually enables the synthesis of ATP. In this electron transport chain, the final acceptor of electrons is molecular oxygen which thereby is reduced to produce water. Occasionally, however, oxygen is prematurely and incompletely reduced, giving rise to superoxide. The superoxide anion belongs to a class of oxygen-derived molecules that readily oxidize other molecules and are commonly referred to as reactive oxygen species (ROS). It has been known for a long time that an excessive load of ROS damages diverse cellular macromolecules, including proteins, lipids, and DNA, a process known as oxidative stress. The concept of oxidative stress has its roots in the mid-20th century when researchers began to explore the harmful effects of oxidizing free radicals (Gerschman *et al.*, 1954) and their possible involvement in the aging process (Harman, 1956). A couple of years later, the antioxidant enzyme superoxide dismutase (SOD) was discovered which eliminates superoxide from the cell and thus protects the cell from its toxicity (McCord *et al.*, 1971). Since then, ROS have been seen as harmful but unavoidable by-products of an aerobic lifestyle.

It therefore came as a surprise when enzymes were discovered whose sole function is the production of ROS (Suh *et al.*, 1999). In fact, these

ROS-generating enzymes, termed NADPH oxidases (NOX) and dual oxidases (DUOX), are present in most eukaryotes (Aguirre and Lambeth, 2010). Therefore, the view that ROS are purely harmful by-products of mitochondrial metabolism needed reconsideration. It is important to note, though, that despite the existence of ROS-producing enzymes, the vast majority of cellular ROS (estimated at approximately 90%) can be traced back to a mitochondrial origin (Balaban *et al.*, 2005). Nevertheless, the fact that ROS are actively synthesized prompted research into their possible biological functions. It is now clear that ROS act as important signaling molecules in diverse physiological processes (Sena and Chandel, 2012). Therefore, organisms must tightly control the balance between ROS production and degradation. This fine-tuned balance between oxidants and antioxidants is called redox homeostasis.

6.3 Arthropod immunity: antimicrobial peptides (AMPs), ROS, and autophagy

The innate immune response of arthropods consists of multiple defense mechanisms, including epithelial barriers and both local and systemic immune reactions. Most research in arthropod immunity has focused on insects, and on *Drosophila melanogaster* in particular (Lemaitre and Hoffmann, 2007; Buchon *et al.*, 2014). Although some components of the arthropod immune system are highly conserved, recent comparative genomic analyses of immune signaling pathways have also revealed a remarkable diversity, even within the insects (Waterhouse *et al.*, 2007; Palmer and Jiggins, 2015). Our discussion of arthropod immunity is largely based on insights from *Drosophila*; however, we will point to divergent findings from other arthropods whenever necessary.

The cellular immune response is executed by hemocytes and emcompasses several distinct mechanisms, including phagocytosis, encapsulation, coagulation, and melanization (Lemaitre and Hoffmann, 2007). Some of these mechanisms (encapsulation, melanization) involve the generation of ROS at infection sites to kill pathogens (Kumar *et al.*, 2003). At the core of the systemic immune response lies the production of antimicrobial peptides (AMPs) by the fat body and their subsequent release into the hemolymph. AMP gene expression is mainly controlled by two distinct signaling pathways, the Toll pathway and the Imd pathway, both of which include homologues of the NF-κB pathway (Lemaitre and Hoffmann, 2007; Hetru and Hoffmann, 2009). The Imd pathway is predominantly

activated by Gram-negative bacteria, whereas Gram-positive bacteria, fungi and yeast trigger the Toll pathway (Buchon *et al.*, 2014). Interestingly, major components of the Imd pathway are missing in chelicerates (Palmer and Jiggins, 2015; Bechsgaard *et al.*, 2016), suggesting that two functionally Imd pathways exist, the canonical one in insects and crustaceans, and an atypical one in chelicerates and myriapods (Shaw *et al.*, 2017).

In the lab, systemic responses have frequently been elicited by bacterial injection into the hemocoel. However, this might not reflect the natural way of infection. Commonly, epithelia such as those lining the gut are the first barrier a pathogen encounters when infecting the host. A peculiarity of gut epithelia is the fact that they not only are in constant contact with pathogens, but also host a number of beneficial commensal bacteria, the so-called gut microbiota. Commensal gut microbes are involved in diverse physiological functions of their hosts (Erkosar *et al.*, 2013; Sommer and Bäckhed, 2013). The challenge for the host immune system, therefore, is to find the balance between fighting pathogens and tolerating the microbiota (Sansonetti and Medzhitov, 2009). Accordingly, a tight regulation of the production of immune effector molecules is strictly needed. In the *Drosophila* gut, there are two major classes of immune effectors, AMPs and ROS (Kuraishi *et al.*, 2013). AMP generation in the gut is controlled by the Imd pathway, but not by the Toll pathway (Tzou *et al.*, 2000). The Imd pathway is triggered when the bacterial cell wall component diaminopimelic acid (DAP)-type peptidoglycan (PG) is recognized by PG recognition proteins (PGRPs) in the host membrane (Leulier *et al.*, 2003; Bosco-Drayon *et al.*, 2012). In the absence of pathogenic bacteria, PG-triggered AMP gene expression is repressed by negative regulators of the Imd pathway to protect the commensal microbiota, thereby maintaining the balance between immune tolerance and immune response (Paredes *et al.*, 2011; Bosco-Drayon *et al.*, 2012; Bonnay *et al.*, 2013).

Local production of AMPs only seems to constitute a complementary response against microbes that are resistant against ROS (Ryu *et al.*, 2006), the second major immune effector class in the *Drosophila* gut. Indeed, DUOX-dependent production of microbicidal ROS serves as the first line of defense in gut immunity (Ha *et al.*, 2005a, 2009a). Importantly, and in contrast to canonical Imd pathway components, DUOX genes were found in species of each arthropod subphylum (Palmer and Jiggins, 2015). Infection-induced ROS generation in the *Drosophila* gut can also act as a signal for AMP production in the fat body, thus triggering a systemic immune response (Wu *et al.*, 2012). After the pathogen-induced increase in ROS production, ROS levels are actively reduced by immune-regulated

catalase (IRC) activity to avoid excessive oxidative stress (Ha *et al.*, 2005b).

DUOX-dependent ROS production in the *Drosophila* gut is regulated by two signaling pathways (Bae *et al.*, 2010): The enzymatic activity of DUOX is controlled by the Gαq-PLCβ-Ca^{2+} pathway ("DUOX activity pathway") (Ha *et al.*, 2009a), while DUOX gene expression is regulated by a MEKK1-MKK3-p38-ATF2 pathway ("DUOX expression pathway") (Ha *et al.*, 2009b; Chakrabarti *et al.*, 2014). Activation of both pathways is required for stable ROS production. Interestingly, PG is able to activate the DUOX expression pathway, but not the DUOX activity pathway. Therefore, DUOX-dependent ROS generation cannot depend on PG alone (Ha *et al.*, 2009a,b; Bae *et al.*, 2010). Recently, bacterial-derived uracil was identified as a non-PG ligand triggering DUOX-dependent ROS generation (Lee *et al.*, 2013). Uracil is probably recognized by a G-protein-coupled receptor (GPCR) and, via Hedgehog-induced signaling endosomes, induces PLCβ-dependent Ca^{2+} mobilization which triggers DUOX activation (Lee *et al.*, 2015). Strikingly, uracil is released by pathogenic bacteria, but not by commensal symbionts (Lee *et al.*, 2013). This allows the gut epithelia to distinguish between pathogens and commensal bacteria, thus maintaining immune homeostasis in the *Drosophila* gut (You *et al.*, 2014).

Arthropods must also fight against intracellular pathogens. In general, host defenses against intracellular pathogens are less well studied than those against extracellular pathogens. Antiviral immunity seems to be based on diverse mechanisms, including RNA interference, activation of the Toll/Imd and JAK-STAT pathways, and autophagy (Lemaitre and Hoffmann, 2007; Sabin *et al.*, 2010; Lamiable and Imler, 2014; Cheng *et al.*, 2016). Autophagy seems to represent a general and evolutionarily conserved defense mechanism against intracellular pathogens (Deretic, 2010; Choy and Roy, 2013; Gomes and Dikic, 2014). In *Drosophila*, for example, one type of PGRP (PGRP-LE) acts as an intracellular receptor for DAP-type peptidoglycan and thus as an intracellular sensor of Gram-negative bacteria (Kaneko *et al.*, 2006). PGRP-LE also induces an autophagic response to prevent the intracellular growth of bacterial pathogens, and this induction occurs independently of the Toll and Imd pathways (Yano *et al.*, 2008). Moreover, autophagy is also activated and regulated by ROS (Huang *et al.*, 2009; Scherz-Shouval and Elazar, 2011; Sena and Chandel, 2012). In sum, several distinct and yet interconnected immune responses are at work to defend the arthropod host against a plethora of different pathogens.

6.4 *Wolbachia* and arthropod immunity

In principle, hosts can employ two different strategies to defend themselves against infections: resistance and tolerance. Resistance is the ability to clear the infection, while tolerance is the ability to reduce the fitness costs of infection (without clearing the infection itself) (Schneider and Ayres, 2008). Whether a host responds to *Wolbachia* through resistance or tolerance strongly depends on two features of the infection: its age and its phenotypic effects. A recently acquired infection is likely to trigger an immune response, which is the key resistance mechanism. In coevolved associations, by contrast, resistance may no longer be the best response to infection. Whether or not resistance is the host's best option in coevolved symbioses mainly depends on the symbiont's phenotype. Reproductive manipulations such as feminization and male killing reduce host fitness and thus are expected to lead to the evolution of resistance. Indeed, host suppressor alleles have been identified that confer resistance to feminizing and male-killing *Wolbachia* (Rigaud and Juchault, 1992; Hornett *et al.*, 2006). With other *Wolbachia* phenotypes, things are a bit more complex. In the case of CI, infected females are 'addicted' to *Wolbachia*—if they lose the symbionts, their offspring will suffer from high mortality rates when fathered by infected males. Therefore, females infected with CI-*Wolbachia* are selected to maintain the bacteria and even increase the efficiency of maternal transmission. On the other hand, suppressor genes are predicted to spread in males, and successive selection for male suppressors of *Wolbachia* should lead to long-term elimination of infection (Koehncke *et al.*, 2009). With respect to *Wolbachia*-induced thelytokous parthenogenesis, the symbiont has gone to fixation in most populations that are infected. In these populations there are no males, and females depend on the bacteria for asexual reproduction. Under such circumstances of host dependence, infected females are not expected to evolve mechanisms of resistance ('dependence' barrier to resistance; see Chapter 4). However, nuclear suppressor alleles have been hypothesized for populations where infected and uninfected individuals coexist (Huigens, 2003). Finally, if *Wolbachia* exhibit a mutualistic phenotype, evolution of resistance will also be selected against ('fitness benefit' barrier to resistance; see Chapter 4). When resistance is not feasible, tolerance mechanisms represent an alternative host strategy to deal with the infection. The evolution of tolerance is associated with the attenuation of the immune response that originally was there to eliminate the bacteria. Immune tolerance is also an efficient means to reduce the risk that host tissue is damaged as a side effect of the immune response (immunopathology).

In summary, the evolution of host resistance is expected in many, but not all, *Wolbachia*–host associations. In those associations in which resistance evolution is expected, *Wolbachia* should, in principle, trigger the host immune system which should aim at eliminating the bacteria, regardless of whether they are novel or native. On the other hand, given the huge number of infected insect species and the recurrent occurrence of successful transmission into novel host species, why is the host defense machinery not more efficient in overcoming the infection? Have *Wolbachia* evolved mechanisms to suppress or interfere with the immune system, or do they hide from it? Or does the high prevalence of *Wolbachia* indicate that, frequently, hosts are not selected to evolve resistance (but rather tolerance)? In the following paragraphs, we outline in more detail the interplay between *Wolbachia* infection and the different host defense mechanisms, with special emphasis on the host oxidative environment.

6.4.1 *Wolbachia* and AMP-/autophagy-based immunity

Interestingly, in their native hosts, *Wolbachia* do not induce AMP gene expression, as has been shown for *Aedes albopictus*, *Drosophila melanogaster*, *D. simulans*, and *Tetranychus urticae* (Bourtzis *et al.*, 2000; Wong *et al.*, 2011; Rancès *et al.*, 2012; Zhang *et al.*, 2015). On the other hand, *Wolbachia*-infected *D. simulans* and *Ae. albopictus* are still able to activate AMP gene expression when challenged by other bacterial pathogens, e.g. *E. coli* (Bourtzis *et al.*, 2000). These results suggest that *Wolbachia* neither induce nor suppress the AMP-based branch of the immune system of their natural hosts. *Drosophila* species seem to be naturally infected with only two maternally inherited bacteria, *Wolbachia* and *Spiroplasma* (Mateos *et al.*, 2006). In *Spiroplasma*-infected *D. melanogaster*, the same picture emerges: in their natural host, the bacteria neither upregulate nor downregulate the expression of AMP genes (Hurst *et al.*, 2003; Hutchence *et al.*, 2011). Taken together, these findings suggest that endosymbionts such as *Wolbachia* have evolved means to evade the host immune system by stealth (Figure 6.1D; Siozios *et al.*, 2008). This notion is corroborated by the fact that, in the host cytoplasm, *Wolbachia* are located within vesicles whose outermost membrane is of host origin (Louis and Nigro, 1989). This probably helps the bacteria to hide from the host immune system. Another possible reason for the lack of *Wolbachia*-induced AMP upregulation is that the host has shut down the AMP-based immune response when selection favors the maintenance of the bacteria (Figure 6.1G). However, it is unclear how this immune tolerance could be restricted to *Wolbachia* so that other pathogens are still effectively

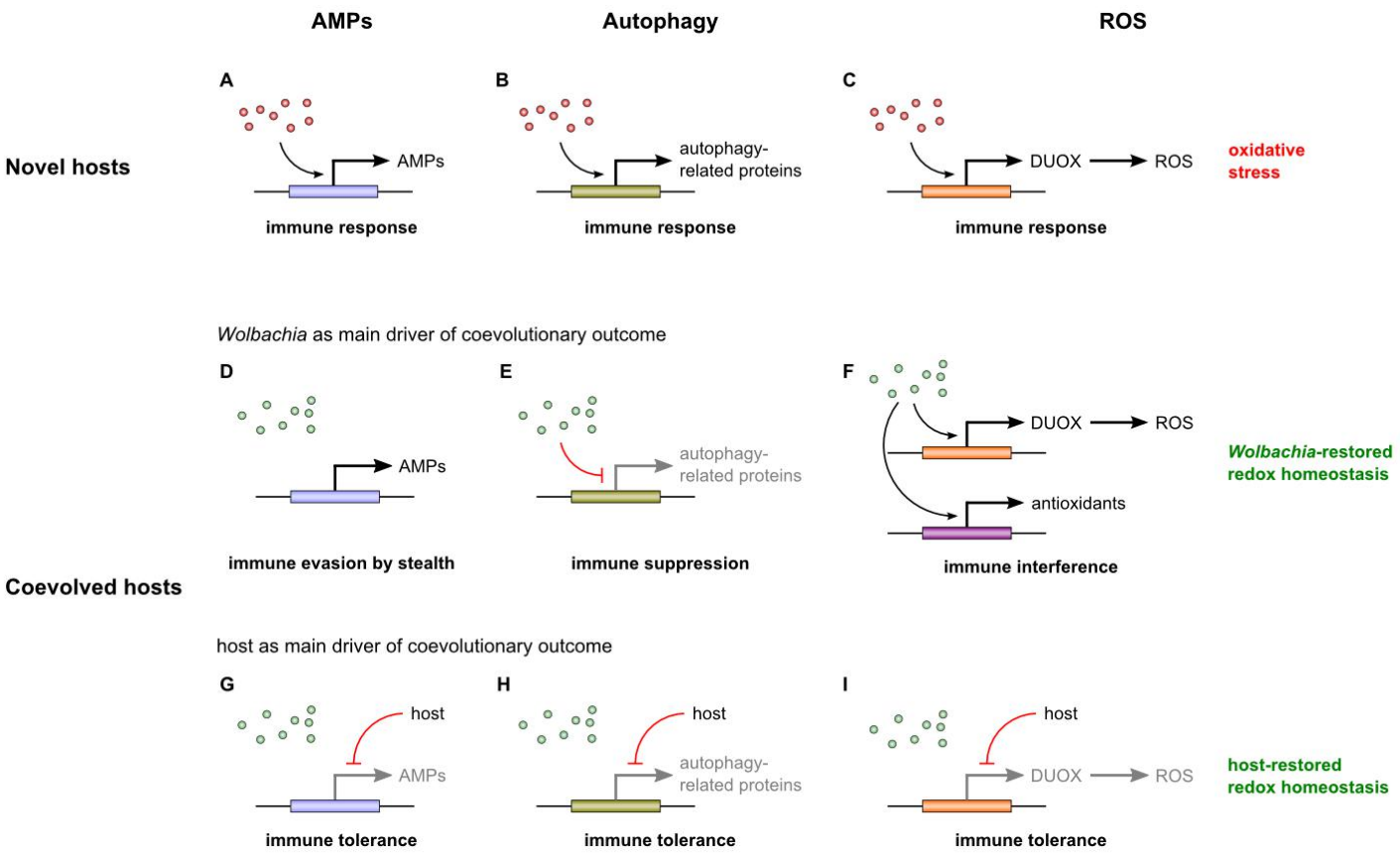

AMPs
Autophagy
ROS
Novel hosts
A
B
C
AMPs
autophagy-related proteins
DUOX
ROS
oxidative stress
immune response
immune response
immune response
Wolbachia as main driver of coevolutionary outcome
D
E
F
AMPs
autophagy-related proteins
DUOX
ROS
antioxidants
Wolbachia-restored redox homeostasis
immune evasion by stealth
immune suppression
immune interference
Coevolved hosts
host as main driver of coevolutionary outcome
G
H
I
host
AMPs
host
autophagy-related proteins
host
DUOX
ROS
host-restored redox homeostasis
immune tolerance
immune tolerance
immune tolerance

Figure 6.1 (previous page): Hypothesized effects of *Wolbachia* on the immune system of novel (A–C) and coevolved hosts (D–I). Newly introduced *Wolbachia* (red dots) trigger an immune response by upregulating the expression of several immune effectors such as AMPs (A), autophagy-related proteins (B), and ROS (C). A ROS-based immune response leads to oxidative stress. Due to host-symbiont coevolution, native *Wolbachia* (green dots) have ceased triggering an immune response. They neither induce nor suppress AMP expression, but evade the AMP-based immune response by stealth (D). Presumably, they downregulate autophagy-related genes (E). With regard to the ROS-based branch of the immune system, we hypothesize that *Wolbachia* not only induce ROS production and oxidative stress, but also the expression of antioxidant genes. By such immune interference, *Wolbachia* restore redox homeostasis (F). Another coevolutionary outcome is host-driven shutdown of the immune response (immune tolerance; G–I). By evolving ROS-associated immune tolerance, the host restores redox homeostasis itself (I). Note that evolution of resistance is also a possible outcome of coevolution, but eventually leads to symbiosis breakdown and therefore is not depicted here.

targeted. This problem could be resolved by the fact that AMPs do not need to be shut down for ensuring immune tolerance in coevolved symbioses, but instead are actively involved in symbiont maintenance (Login *et al.*, 2011).

The fact that *Wolbachia* do not elicit an AMP-based immune response in their native hosts stands in stark contrast to the strong induction of AMP gene expression when *Wolbachia* are introduced into novel hosts (Figure 6.1A; Xi *et al.*, 2008; Moreira *et al.*, 2009; Kambris *et al.*, 2009, 2010; Bian *et al.*, 2010). This is indicative of a systemic immune response triggered by the canonical Toll and/or Imd pathway (immune upregulation; note that the term immune priming is equivalent to such general immune upregulation only in its unspecific meaning; see, for example, Roth *et al.*, 2009 and Masri and Cremer, 2014 for a different usage of the term). As Gram-negative bacteria, newly introduced *Wolbachia* are probably detected by the Imd pathway that is triggered by recognition of diaminopimelic acid (DAP)-type peptidoglycan from the bacterial cell wall. Although *Wolbachia* lack a proper cell wall and peptidoglycan has never been detected, they are probably able to synthesize DAP (Dunning Hotopp *et al.*, 2006; Vollmer *et al.*, 2013). Moreover, it was recently shown that the peptidoglycan-associated lipoprotein (PAL) is located on the cell membrane of *Wolbachia* (Voronin *et al.*, 2014). PAL is known to specifically bind DAP (Parsons *et al.*, 2006). Therefore, DAP is present on the *Wolbachia* membrane, and perhaps this is

sufficient to be recognized by peptidoglycan recognition proteins (PGRPs) which then trigger the Imd pathway and subsequent AMP generation.

The discovery of PGRP-LE as an intracellular sensor of DAP-type peptidoglycan Kaneko *et al.* (2006) also opens the possibility of an autophagic immune defense against *Wolbachia*. It was recently shown that *Wolbachia* induce the autophagy pathway in a naturally infected *Aedes albopictus* cell line (Voronin *et al.*, 2012). Hence, one might expect bacterial strategies to counteract autophagy. Indeed, autophagy-associated genes are downregulated in the ovaries of two hosts naturally infected with *Wolbachia*, the woodlouse *Armadillidium vulgare* and the wasp *Asobara tabida*, supporting the notion that the symbionts suppress the autophagic signal to prevent their elimination (Figure 6.1E; Chevalier *et al.*, 2012; Kremer *et al.*, 2012. Again, it is also conceivable that the host itself is responsible for the downregulation – another possible case of evolved immune tolerance when symbiont presence is favored (Figure 6.1H). In contrast to these coevolved associations, a transfected *Wolbachia* strain causes a catastrophic autophagic response in another woodlouse, *Porcellio d. dilatatus*, resulting in the death of the new host (Figure 6.1B; Le Clec'h *et al.*, 2012). Therefore, an autophagic immune response is observable in novel, but not in native hosts, mirroring the situation with regard to AMP-based immune defense.

How can we reconcile these differing findings concerning the immune response to *Wolbachia* in native vs. novel hosts? Perhaps, it is not too surprising that *Wolbachia* do elicit an immune response in novel hosts. In insects that acquired *Wolbachia* only recently (either by natural or artificial means), the bacteria are recognized as foreign, probably by PGRPs detecting DAP on *Wolbachia* membranes, and then AMP- and/or autophagy-associated defense mechanisms are triggered to eliminate the infection (Figure 6.1A, B). With ongoing coevolution, however, *Wolbachia* have found ways to prevent their elimination, for example by evading the AMP-based immune response (immune evasion by stealth; Figure 6.1D) and by suppressing the autophagy-associated immune defense (immune suppression; Figure 6.1E). Alternatively, evolution of immune tolerance enables the host to reduce costly defense mechanisms when selection favors the presence of *Wolbachia* (Figure 6.1G, H).

6.4.2 *Wolbachia* and ROS-based immunity

Given their vertical transmission through the female germline and their reproductive manipulations, *Wolbachia* are expected to reside primarily in the host reproductive tissues. Although this is true, they are also able to

infect somatic tissues, including tissues of immunological importance, such as the gut, Malpighian tubules, fat body, and hemolymph (Dobson *et al.*, 1999; Cheng *et al.*, 2000; Ijichi *et al.*, 2002; Goto *et al.*, 2006; Zouache *et al.*, 2009; Faria and Sucena, 2013; Frost *et al.*, 2014; Braquart-Varnier *et al.*, 2015; Pietri *et al.*, 2016). In gut epithelia, AMPs represent only one of two major classes of immune effectors, the other one being ROS (see Section 6.3). Therefore, when asking about the relationship between *Wolbachia* infection and the host immune system, it is also important to consider possible interactions between *Wolbachia* on the one hand and ROS and the oxidative environment on the other hand, in particular if infection in the gut has been reported.

The first, indirect evidence of such an interaction between *Wolbachia* and the host oxidative environment came from studies on the role of mitochondria in various aspects of host biology. In *Drosophila melanogaster*, tetracycline treatment to eliminate *Wolbachia* resulted in a significant decrease in lipid hydroperoxide, a marker for ROS-induced oxidative damage (Driver *et al.*, 2004). However, this finding could be due to a direct negative effect of tetracycline on mitochondrial efficiency (see Section 4.5). Further indirect evidence comes from the fact that *Wolbachia* infection can have a profound influence on mitochondrial DNA (mtDNA) haplotype diversity (Hurst and Jiggins, 2005), and different mtDNA haplotypes can differ in mitochondrial ROS production rates (Ballard, 2005).

Brennan *et al.* (2008) were the first to demonstrate a more direct effect of *Wolbachia* on the host oxidative environment. The mosquito *Aedes albopictus* is naturally infected with CI-inducing *Wolbachia*. In an *Ae. albopictus* cell line, the authors found that *Wolbachia* infection is associated with high levels of ROS (as compared to an identical cell line treated with the antibiotic rifampicin). These ROS probably are a product of the host immune response (although they may also be a side-product of bacterial metabolism). In addition, *Wolbachia* infection is associated with the upregulation of several host antioxidant genes. These antioxidant proteins include copper-zinc superoxide dismutase (SOD1), peroxiredoxin (Prx5), and glutathione peroxidase (GPx). Antioxidant upregulation may be a host countermeasure to mitigate the negative effects of increased ROS levels. However, as the authors point out, there is so far only little support in the literature for antioxidant upregulation as a host response to pathogen infection. Alternatively, one might speculate that *Wolbachia* induce the host antioxidant system in order to be protected against the host immune response based on increased ROS levels. Possibly, induction of the host antioxidant system is due to effectors secreted by the bacterial type

IV secretion system (T4SS). The *Wolbachia* T4SS is a potential pathway to transfer effector proteins into the host cytoplasm and therefore might be involved in *Wolbachia*-induced host phenotypes (Pichon *et al.*, 2009). Recently, a T4SS effector in Ehrlichia (a close relative of *Wolbachia*) was shown to be translocated to mitochondria and to upregulate a host SOD (MnSOD), thereby reducing ROS levels and apoptosis (Liu *et al.*, 2012). Lastly, *Wolbachia* also seem to be able to produce their own antioxidants to protect themselves, since two bacterial antioxidant proteins were identified as well, a bacterial type of SOD (Fe-SOD) and bacterioferritin (Bfr). Iron (Fe) is an essential element for most organisms, but also a cause of oxidative stress as it catalyzes the generation of highly reactive hydroxyl radicals (Fenton reaction) (Nappi and Vass, 2002). Hence, bacterioferritin has important functions both in bacterial iron storage and, although not commonly referred to as an antioxidant, in fighting iron-mediated oxidative stress (Carrondo, 2003). Upregulation of *Wolbachia* bacterioferritin expression under iron-induced stress was also observed in naturally infected *Drosophila simulans* (Kremer *et al.*, 2009b). Given the crucial role of iron at the interface of immunity, infection and host-pathogen interactions (Cassat and Skaar, 2013; Nairz *et al.*, 2014), *Wolbachia*'s ability to interfere with host iron metabolism might be an important factor underlying diverse phenotypes and thus contributing to the unparalleled success of *Wolbachia* (Gill *et al.*, 2014).

Wolbachia and the oxidative environment: a hypothesis

Based on the results by Brennan *et al.* (2008), on subsequent propositions regarding the possible involvement of *Wolbachia* in the host oxidative environment (Kremer *et al.*, 2010; Moné *et al.*, 2014), and on the findings concerning AMP-/autophagy-based immunity, we propose the following hypothesis. In novel hosts, *Wolbachia* induce a ROS-based immune response, leading to oxidative stress (Figure 6.1C). In general, therefore, we expect infections in novel hosts to be associated with a disruption of redox homeostasis (although *Wolbachia* effects on antioxidant production are hardly predictable). In native hosts, by contrast, *Wolbachia* infection is expected to be associated with restored redox homeostasis, resulting from coevolutionary processes between symbiont and host. Redox homeostasis can be restored by *Wolbachia* or by the host (or by a combination of both) because both benefit from reduced oxidative stress. In the first case, *Wolbachia* not only induce a ROS-based immune response, but also the expression of antioxidant genes (regardless of whether these genes are part of the symbiont or host

genome). In doing so, the bacteria interfere with the host immune response (immune interference) and are involved in maintaining redox homeostasis (Figure 6.1F). This may be particularly relevant if there are additional sources of oxidative stress (e.g., iron overload). In the second case, the host decreases the *Wolbachia*-induced immune response by reducing ROS production or by increasing antioxidant production (immune tolerance), and thereby restores redox homeostasis itself (Figure 6.1I). In what follows, we will gather further evidence in support of this hypothesis.

Wolbachia and the oxidative environment in novel hosts

Several studies report effects of *Wolbachia* infection on the oxidative environment of arthropods that are naturally either uninfected or infected with a different strain. Examples where a novel *Wolbachia* infection causes an increase in ROS levels include the mosquitoes *Aedes aegypti* (Pan *et al.*, 2012), *Ae. polynesiensis* (Andrews *et al.*, 2012) and *Anopheles stephensi* (Bian *et al.*, 2013). All mosquitoes were transfected with the *Wolbachia* strain wAlbB, which naturally infects *Aedes albopictus*. In *Ae. aegypti*, ROS production was shown to be due to the upregulation of NADPH oxidase (NOXM) and dual oxidase (DUOX2), the latter one being upregulated 28-fold (Pan *et al.*, 2012). Interestingly, the authors found that increased ROS levels activate the Toll pathway, leading to the production of AMPs and antioxidants. The fact that *Wolbachia* induce both the activation of ROS and antioxidants in *Ae. aegypti* is reminiscent of the situation in evolved symbioses (immune interference by *Wolbachia*). Given the relatively close phylogenetic relationship between donor (*Ae. albopictus*) and recipient (*Ae. aegypti*), it might not be too difficult for *w*AlbB to induce antioxidant production in *Ae. aegypti* and thus establish redox homeostasis in a novel host. On the other hand, some studies involving transfected cell lines show the downregulation of antioxidants as a result of infection (Xi *et al.*, 2008; Hughes *et al.*, 2011c). In sum, there is good evidence of the induction of ROS production by *Wolbachia* in novel hosts, whereas findings on the effects of novel infections on antioxidant production are so far inconclusive.

Wolbachia and the oxidative environment in native hosts

In addition to the results by Brennan *et al.* (2008) in an *Aedes albopictus* cell line, there is also evidence in support of our hypothesis that comes from whole insects. Using different methods, Molloy and Sinkins (2015) re-examined the production of ROS and antioxidants in *Ae. albopictus*, both

in mosquito and cell lines. Interestingly, they did not find any significant difference in infected vs. uninfected lines, a finding that differs from that by Brennan *et al.* (2008). Nevertheless, it can similarly be interpreted as an outcome of host-symbiont coevolution, i.e. as attentuation of the immune response to *Wolbachia* in its natural host (immune tolerance). Thus, although it is unclear why both studies come to different results at the molecular level, the conclusion that can be drawn from them is the same: coevolution between *Aedes albopictus* and its *Wolbachia* las led to restored redox homeostasis, either through immune interference (suggested by the results from Brennan *et al.*, 2008) or immune tolerance (suggested by Molloy and Sinkins, 2015).

In *Drosophila simulans* naturally infected with *Wolbachia*, total ROS levels are significantly higher in infected males than in males cured of infection. Moreover, DUOX is located in close proximity to the *Wolbachia*-containing vesicles (Haukedal, 2013). This suggests that the host recognizes *Wolbachia* as foreign and prompts an immune response involving DUOX-dependent ROS production. On the other hand, total SOD levels (including two host SODs and bacterial Fe-SOD) are also significantly higher in infected flies than in uninfected flies (Brennan *et al.*, 2012). These findings suggest that *Wolbachia* infection in the natural host *D. simulans* induces not only a host immune response, but also antioxidant production.

As already mentioned, the ability of *Wolbachia* to interfere with the host oxidative environment might be of particular importance if the level of oxidative stress is elevated by external factors. Toxicity of the heavy metal lead is mainly attributed to its ability to generate ROS and to impair the antioxidant defense (Flora *et al.*, 2012). When *Drosophila melanogaster* is challenged by a lead-contaminated diet, flies cured of infection exhibit a strongly increased malondialdehyde content, which is a marker for oxidative stress. In addition, high-lead diet significantly decreases SOD activity in cured flies, but not in infected flies (Wang *et al.*, 2012).

Another example of the putative role of *Wolbachia* in maintaining redox homeostasis under stressful conditions involves the oxidative challenge imposed by blood-feeding. Ingestion of a blood meal is associated with the release of large amounts of the iron-containing cofactor heme in the gut. When not bound to proteins, heme has potential pro-oxidant and cytotoxic effects in that it converts weakly reactive oxygen species into highly reactive ones (Jeney *et al.*, 2002)). Hematophagous insects have evolved different mechanisms to be protected from these cytotoxic effects, including the binding, aggregation, and degradation of heme, and expression of antioxidant enzymes (Oliveira *et al.*, 1999; Paiva-Silva *et al.*, 2006;

Graça-Souza *et al.*, 200). Maintenance of redox homeostasis in the midgut after a blood meal is crucial, not least because of the pivotal role of ROS in gut immunity. In the mosquito *Aedes aegypti*, a blood meal leads, perhaps counterintuitively at first, to a dramatic decrease in ROS levels in the midgut (Oliveira *et al.*, 2011). This decrease is due to a heme-mediated activation of protein kinase C (PKC) which leads to lowered ROS generation in midgut epithelial cells. The authors interpret this as an adaptation to compensate for the pro-oxidant blood meal and to avoid heme-mediated oxidative stress, thus maintaining redox homeostasis. However, lowered ROS levels in the gut are probably associated with decreased resistance to infection and increased mortality (Oliveira *et al.*, 2011). Interestingly, overall ROS levels do not change significantly after a blood meal in *Aedes polynesiensis* which, unlike *Ae. aegypti*, is naturally infected with *Wolbachia* (Andrews *et al.*, 2012). In a coevolutionary process, the host might have abolished the PKC-mediated decrease in ROS levels in the gut (tolerance) because of *Wolbachia*-induced antioxidant production (immune interference). Therefore, it is reasonable to assume that *Wolbachia* help in maintaing redox homeostasis in this evolved symbiosis (Gill *et al.*, 2014). Moreover, when *Ae. polynesiensis* is fed sucrose only, there is no significant difference between ROS levels of infected and cured mosquitoes, and these ROS levels are lower than that of artificially infected mosquitoes (Andrews *et al.*, 2012). A possible explanation for this finding is that, due to coevolution between *Ae. polynesiensis* and its symbiont, the mosquito has reduced ROS production to mitigate oxidative stress (immune tolerance). Evolution of immune tolerance might therefore also be at play in the *Wolbachia–Ae. polynesiensis* symbiosis (Moné *et al.*, 2014).

In the spider mite *Tetranychus urticae*, *Wolbachia* infection is associated with the enrichment of gene sets related to oxidoreductase activity (Zhang *et al.*, 2015). Oxidoreductases are known to produce ROS (Raha and Robinson, 2000; Esterházy *et al.*, 2008), but also to control redox homeostasis (Messens *et al.*, 2013). Moreover, *Wolbachia* encodes an oxidoreductase (α-DsbA1) which, due to its low redox potential, might have antioxidant properties (Kurz *et al.*, 2009). Therefore, it is conceivable that *Wolbachia* directly or indirectly regulate redox homeostasis and thus maintain their association with *T. urticae*.

We do not want to conceal that there also are some findings from natural *Wolbachia*–host associations that are more difficult to reconcile with the above hypothesis, or at least more difficult to interpret. In the pill bug *Armadillidium vulgare*, some antioxidants (thioredoxin, ferritin) are upregulated in the ovaries of infected individuals (as compared to uninfected ones),

while others (peroxiredoxin, glutathione peroxidase) are downregulated (Chevalier *et al.*, 2012). Perhaps, upregulation of some antioxidants is just a compensation for the downregulation of others, or vice versa. It is unclear, however, what is induced by the host and what by the bacteria. In the parasitoid wasp *Asobara tabida*, the expression of several antioxidant genes (oxidoreductase, glutathione peroxidase, ferritin) is downregulated in infected ovaries, compared to ovaries from cured wasps (Kremer *et al.*, 2012). At first, this seems to contradict our hypothesis. However, the *Wolbachia–A. tabida* association is a special case because here, the host is strictly dependent on its symbiont (see Section 4.4.2). Females cured of infection fail to produce oocytes, due to extensive apoptosis in egg chambers (Pannebakker *et al.*, 2007). The authors suggest a coevolutionary scenario where the wasp responds to infection with apoptosis, which is then suppressed by *Wolbachia*. *A. tabida* in turn compensates for suppression by further increasing the apoptotic signal because it is essential for proper egg development (Pannebakker *et al.*, 2007). There is good empirical support for this scenario. First, *Wolbachia* are probably able to directly or indirectly suppress apoptosis. Suppression of apoptosis might be due to *Wolbachia* interfering with host iron metabolism and oxidative stress control (Kremer *et al.*, 2010; Gill *et al.*, 2014; see Chapter 4). Moreover, it is known that ROS can act as initiators and mediators of apoptosis (Simon *et al.*, 2000; Dixon and Stockwell, 2014). Therefore, downregulation of antioxidant genes could be a host measure to further increase the apoptotic signal. In sum, the dependence of *A. tabida* on *Wolbachia* might well be a consequence of the evolution of tolerance following the disruption of redox homeostasis (Moné *et al.*, 2014; see Chapter 4).

Lastly, we point to the fact that all cases that are compatible with the hypothesis involve CI-inducing *Wolbachia* (*Aedes albopictus*, *Ae. polynesiensis*, *Drosophila melanogaster*, *D. simulans*, *Tetranychus urticae*). In contrast, *Armadillidium vulgare* is naturally infected with feminizing *Wolbachia*, and the strain that *Asobara tabida* depends on for oogenesis does not seem to exhibit any reproductive phenotype (although the possibility that it induces CI remains untested). Therefore, one could think of a mechanistic connection between the host oxidative environment and the CI phenotype. Indeed, Brennan *et al.* (2012) showed that total SOD levels are significantly higher in testes of *D. simulans* males infected with CI-*Wolbachia* than in testes of cured males. Taking this as evidence of higher oxidative stress in infected testes, the authors presumed that disruption of redox homeostasis caused DNA damage in spermatocytes of infected males. Strikingly, DNA damage is significantly higher in infected compared to uninfected spermatocytes and

might be a contributing factor to the sperm modification characteristic of CI (Brennan *et al.*, 2012). For example, DNA damage in spermatocytes could, after fertilization, lead to DNA replication defects in the male pronucleus as observed in CI crosses in *D. simulans* (Landmann *et al.*, 2009).

6.4.3 *Wolbachia* and anti-pathogenic effects

The possibility of *Wolbachia*-induced host protection has recently spurred intense research efforts. Taking a slightly critical stance, in Chapter 4, we proposed to distinguish protection from mere anti-pathogenic effects. Following our definition, *Wolbachia* are said to induce an anti-pathogenic effect whenever infection increases host resistance and/or tolerance to pathogens. However, an anti-pathogenic effect should only be classified as protection if it is associated with a fitness benefit to the host. Since so far only a few studies have found evidence for *Wolbachia*-mediated protection in the field (Hedges *et al.*, 2008; Teixeira *et al.*, 2008; Osborne *et al.*, 2009; Zélé *et al.*, 2012), we here focus on anti-pathogenic effects.

The molecular mechanisms underlying *Wolbachia*-mediated anti-pathogenic effects are still unclear (Rainey *et al.*, 2014; Johnson, 2015; see Chapter 4). Antiviral effects seem to be more frequent than antibacterial effects. Moreover, the strength of the anti-pathogenic effect is positively correlated to *Wolbachia* density. But how is the insect immune system involved? Anti-pathogenic effects are frequently observed when *Wolbachia* are transfected into hosts that are either naturally uninfected or infected with a different strain. As outlined above, in such cases, infection induces the upregulation of host immune genes, in particular genes involved in the Toll and Imd pathway, leading to the generation of AMPs. Such immune upregulation of Toll/Imd pathway genes is assumed to underlie anti-pathogenic effects in novel hosts, especially antiviral effects in mosquitoes (Xi *et al.*, 2008; Moreira *et al.*, 2009; Kambris *et al.*, 2009, 2010; Bian *et al.*, 2010; Pan *et al.*, 2012). However, other studies have shown that, both in native and novel hosts, genes involved in the Toll or Imd pathway are not required for *Wolbachia*-mediated anti-pathogenic effects (Wong *et al.*, 2011; Rancès *et al.*, 2012, 2013; Chrostek *et al.*, 2014; Ferreira *et al.*, 2014; Martinez *et al.*, 2014). Therefore, upregulation of immune genes involved in the Toll/Imd pathways cannot be the universal explanation for *Wolbachia*-induced anti-pathogenic effects, let alone for host protection in the field (see Chapter 4).

The possible role of ROS in *Wolbachia*-induced anti-pathogenic effects has been less intensively studied than that of AMPs. The mosquito *Aedes aegypti*

is naturally not infected with *Wolbachia*, but transfection of the wAlbB strain into *Ae. aegypti* inhibits replication of Dengue virus (Bian *et al.*, 2010). It could be shown that transfection induces NOX- and DUOX-dependent ROS generation. Increased ROS levels activate the Toll pathway, which then mediates the production of antioxidants and AMPs such as defensin and cecropin. These AMPs are involved in inhibiting the proliferation of Dengue virus in *Wolbachia*-transfected mosquitoes (Pan *et al.*, 2012). In transfected *Ae. albopictus* mosquitoes, by contrast, ROS-mediated immune activation is probably not involved in the antiviral effect of *Wolbachia* (Molloy and Sinkins, 2015). A recent study analyzed the relationship between ROS levels and antiviral effects in naturally infected *Drosophila* strains (Wong *et al.*, 2015). The study included *Wolbachia* strains that were known to either have an anti-pathogenic effect ('protective' strains) or not ('non-protective' strains). In flies that harbor a protective strain, ROS levels are significantly higher than in flies cured of the protective strain. By contrast, presence of the non-protective strain has no significant effect on ROS levels relative to cured flies. These findings suggest that ROS levels are increased in *Drosophila* naturally infected with protective *Wolbachia* strains. Moreover, elevated ROS levels confer a survival advantage against mortality induced by *Drosophila* C virus (DCV) (Wong *et al.*, 2015). The anti-DCV effect is probably not mediated by the Toll pathway because *Wolbachia*-induced antiviral effects were shown to be independent of this pathway in *Drosophila* for both Dengue virus and DCV (Rancès *et al.*, 2013; Ferreira *et al.*, 2014). Interestingly, the ROS-mediated survival advantage is not associated with reduced virus accumulation, pointing to increased tolerance rather than resistance (Wong *et al.*, 2015). Tolerance mechanisms have been shown to be at play in other coevolved *Wolbachia*–host systems where the symbionts induce anti-pathogenic effects (Teixeira *et al.*, 2008; Osborne *et al.*, 2009; Zélé *et al.*, 2014). In sum, the possibility that a *Wolbachia*-induced ROS-based immune response is involved in anti-pathogenic effects constitutes a promising topic for future research.

6.4.4 *Wolbachia*, ROS, life-history trade-offs, and mitohormesis

Organisms cannot maximize all fitness-relevant traits at once. Rather, they face the challenge to optimally allocate limited resources among those traits. Hence, the evolution of fitness-related traits is constrained by the existence of trade-offs between them. These trade-offs play a fundamental role in life-history theory (Stearns, 1989). Along these lines, immune defense can be viewed as a life-history trait as well, and trade-offs between immunity and

other fitness-related traits ('costs of immunity') have been gaining increasing attention among evolutionary ecologists (Sheldon and Verhulst, 1996; Zuk and Stoehr, 2015; Schmid-Hempel, 2003; Schulenburg *et al.*, 2009; McKean and Lazzaro, 2011; Schwenke *et al.*, 2016).

Much effort has been made to elucidate the physiological mechanisms underlying life-history trade-offs. Given their antagonistic and pleiotropic effects, ROS have recently been proposed as central players in the occurrence of such trade-offs (Monaghan *et al.*, 2009; Dowling and Simmons, 2009; Metcalfe and Alonso-Alvarez, 2010; Isaksson *et al.*, 2011; but see Speakman and Garratt, 2014). In particular, because of their pivotal role in innate immunity on the one hand and in oxidative stress on the other hand, ROS may be a key factor underlying the trade-off between immunity and other life-history traits such as fecundity and longevity (Moné *et al.*, 2014).

Building upon these ideas and on the intimate connections between *Wolbachia* and the host oxidative environment, one may speculate that *Wolbachia* are involved in the occurrence of the trade-off between immunity and other life-history traits, and that this involvement is, at least in part, mediated by ROS. There is some evidence for this hypothesis. Pigeault *et al.* (2014) studied the effect of transfected *Wolbachia* strains on immunity and reproduction in the woodlouse *Porcellio dilatatus*. They found a clear trade-off between both life-history traits: the *w*Con strain increases investment in immune parameters but reduces reproductive investment (whereas the *w*Dil strain has the converse effect). However, the tested immune parameters (such as hemocyte density or phagocytosis activity) do not allow to draw a conclusion on whether ROS are involved in the trade-off. In *Drosophila simulans*, there is a similar trade-off between *Wolbachia*-induced antiviral protection and egg hatch rates, female fecundity and male fertility (Martinez *et al.*, 2015). Another example of *Wolbachia*-associated costs of immunity involves the trade-off between immunity and longevity. *Wolbachia* strains that induce strong antiviral effects in *Drosophila melanogaster* (so-called *w*MelCS-like strains) often shorten the host lifespan (Chrostek *et al.*, 2013). Strikingly, the *w*MelCS strain was recently shown to increase ROS concentration 2-fold relative to a *Wolbachia*-free control (Wong *et al.*, 2015). Therefore, it is possible that elevated ROS levels are responsible not only for the antiviral effect, but also for the shortened lifespan.

The impact of ROS and oxidative stress on longevity and aging has been debated for more than half a century. The seminal 'free radical theory of aging' states that the production of mitochondrial ROS is the major cause of aging (Harman, 1956, 1972; Balaban *et al.*, 2005). However, findings

are accumulating that seem to be incompatible with this theory (Lapointe and Hekimi, 2010; Speakman and Selman, 2011; Stuart *et al.*, 2014, but see Kirkwood and Kowald, 2012). In particular, recent evidence suggests that moderately increased formation of ROS in the mitochondria causes higher stress resistance and eventually extends life span, a process that has been termed mitochondrial hormesis (mitohormesis) (Ristow and Schmeisser, 2014; Yun and Finkel, 2014). In general, hormesis can be defined as an adaptive response that is characterized by a beneficial effect at low doses and a harmful effect at higher doses. In a narrower, and recently more frequently used, sense, hormesis describes the phenomenon that a mild, sublethal stress causes an adaptive response that protects against larger subsequent stresses. The latter meaning of the term has been named 'stress-response hormesis' (Gems and Partridge, 2008). Mitohormesis represents a form of stress-response hormesis: Mild mitochondrial stress increases ROS formation which induces stress response mechanisms (such as antioxidant production), ultimately causing a long-term reduction of oxidative stress. Mitohormesis thus involves both an increase in mitochondrial ROS and a subsequent antioxidant response, and the notion of a mitohormetic pathway is tightly associated with the role of ROS as important signaling molecules (Hamanaka and Chandel, 2010; Finkel, 2011). Several recent studies have shown this mitohormetic pathway to be at work in promoting survival and longevity (Kharade *et al.*, 2005; Chávez *et al.*, 2007; Schulz *et al.*, 2007; Zarse *et al.*, 2012; Mouchiroud *et al.*, 2013; De Haes *et al.*, 2014; Hirose *et al.*, 2016). Given that *Wolbachia* are known to promote longevity in several hosts (see Chapter 4), it is tempting to speculate that they do so by triggering the mitohormetic pathway. More generally, the mitohormetic pathway is strongly reminiscent of the hypothesized 'immune interference' phenotype of *Wolbachia* in native hosts (in which the symbionts not only induce a ROS-based immune response, but also the expression of antioxidant genes; Figure 6.1F). Taken together, some fitness-enhancing effects of native *Wolbachia* (e.g. promoting longevity, maintaining redox homeostasis) might be attributable to mitohormesis.

With regard to the impact of ROS on fitness-related traits, the trade-off approach and the mitohormesis approach might appear to come to quite different conclusions. For example, ROS are assumed to shorten lifespan under the former approach and to extend lifespan under the latter. More generally, the trade-off approach states that *Wolbachia* (via ROS) have a positive effect on some fitness parameters and a negative effect on others, whereas the mitohormesis approach emphasizes the positive fitness effect of *Wolbachia*-induced mitochondrial ROS formation. However, hormesis itself

is assumed to trade off with at least some fitness-related traits because a positive hormetic effect on overall fitness would be at odds with life-history theory (Forbes, 2000). Accordingly, a recent study finds that pathogen challenge in *Drosophila* enhances not only survival and fecundity, but also susceptibility to infection, suggesting a trade-off between hormesis and immunity (McClure *et al.*, 2014). Therefore, both approaches involve some form of trade-off and thus are not mutually exclusive.

6.5 Conclusion

Reactive oxygen species (ROS) represent a double-edged sword: They are known to cause oxidative stress and damage cellular macromolecules. However, given their cytotoxic nature, ROS also are efficient microbicidal effectors which play a crucial role in the insect immune system. Due to this antagonistic pleiotropy, ROS probably underlie evolutionary trade-offs between immunity and other life-history traits such as fecundity and longevity. *Wolbachia* are widespread intracellular bacteria famous for their ability to modulate exactly these fitness-related host traits in intriguing ways. At the same time, they must be able to cope with the host immune system in order to invade and persist in their insect hosts. Therefore, the host oxidative environment represents a promising area to elucidate the mechanisms of *Wolbachia*–host interactions.

In newly infected hosts, *Wolbachia* usually trigger an immune response which is aimed at eliminating the infection. In coevolved associations, by contrast, either the host has curbed the immune response when it pays to do, or the symbionts have evolved ways to resist the host immune response. They do so by adopting a variety of strategies, including immune evasion by stealth, suppression, and interference. We propose that in coevolved symbioses, *Wolbachia* frequently make use of the latter strategy in that they not only induce a ROS-based immune response but also an antioxidant response. Thereby the bacteria are involved in maintaining redox homeostasis. Interference with the host oxidative environment might also underlie other mutualistic phenotypes of *Wolbachia* such as enhancing host defense or promoting longevity, possibly via mitohormetic effects. On the other hand, *Wolbachia*-induced ROS formation might be involved in parasitic phenotypes such as cytoplasmic incompatibility. Taken together, *Wolbachia*'s impact on the host oxidative environment probably contributed to their tremendous success and opens up exciting avenues for future research.

7 Conclusion and outlook

The main objective of this thesis was to analyze the significance of horizontal transmission and mutualistic effects for the unprecedented success of *Wolbachia* in the global arthropod community. We started our endeavor by quantifying this success, in terms of the number of infected host species. To estimate the infection frequency of *Wolbachia* among arthropods, we used a statistical approach based on a survey that, in contrast to many others, satisfies several criteria for a reliable estimate (no one-individual samples, no excessively large samples, no restriction to a specific host taxon). We found that approximately 40% of all terrestrial arthropod species are infected, implying that millions of species harbor the bacteria. In addition, we estimated the incidence of several other reproductive manipulators and found *Wolbachia* to be by far the most abundant ones (Chapter 2).

This massive number of infections calls for explanation. From an epidemiological perspective, transmission between hosts must compensate for infection loss within hosts, and the *Wolbachia* pandemic is no exception. Indeed, in an epidemiological network model in which *Wolbachia* move between host species (instead of individuals), we found that the ratio between acquisition and loss of infections among species is the major determinant of *Wolbachia* incidence. Moreover, in host communities with an evolutionarily ancient *Wolbachia* infection, incidence is likely to be at equilibrium, whereas the incidence of younger infections might still be increasing on an evolutionary timescale. Lastly, we showed that transmission over large phylogenetic distances is crucial for obtaining high incidence levels (Chapter 3).

Although our model does not explicitly assume beneficial effects of *Wolbachia* infection, such effects are likely to be very helpful for invasion into novel hosts. To assess the scope and diversity of *Wolbachia*-associated host benefits, we performed a thorough investigation of *Wolbachia*–arthropod mutualisms. We found that facultative mutualisms arise either through selection on *Wolbachia* to increase female fitness or through byproduct benefits, i.e. without selection to directly benefit hosts. For example, our comprehensive review of *Wolbachia*-induced anti-pathogenic effects revealed that frequently such pathogen interference is only a byproduct of other processes (e.g. competition for limited resources or triggering of the host

immune system). Moreover, only few studies have so far examined whether pathogen interference actually provides host benefits in the field.

Obligate mutualisms arise through the evolution of dependence, either via host compensatory mechanisms (tolerance) or via the takeover of some host function by *Wolbachia*. Many obligate mutualisms evolve in the context of host reproductive processes, e.g. oogenesis and sex determination. In general, we found many cases in which *Wolbachia* mutualisms, both of the obligate and facultative type, go hand in hand with reproductive parasitism (so-called 'Jekyll and Hyde' infections). We also found that some phenotypes of mutualism and parasitism are likely to share common mechanisms. In sum, our analysis suggested that *Wolbachia* mutualisms can have far-reaching consequences for the relationships between the symbionts and their arthropod hosts (Chapter 4).

In order to analyze the effects of host benefits on the evolution of *Wolbachia*, we modeled the infection dynamics of CI- and MK-inducing symbionts that additionally increase host fitness. We showed that a symbiont's 'effective fitness' (i.e. the product of maternal transmission efficiency and relative fitness of an infected female) is crucial for its invasion success. Our findings corroborate that host benefits substantially facilitate invasion into new populations, and that they are even needed for invasion if CI or MK is weak. The facilitating effect of host benefits also pertains to multiple *Wolbachia* infections with different reproductive manipulations. To show this, we derived, for the first time, invasion conditions and equilibrium frequencies for CI+MK double infections. We then discussed the significance of host benefits for the long-term evolutionary dynamics of CI and MK and their potential role in resolving genetic conflicts between *Wolbachia* and their hosts (Chapter 5).

Finally, we addressed the interrelationships between *Wolbachia* and the arthropod immune system. Just like any other bacterial infection, *Wolbachia* must cope with the host immune system in order to spread. In our review, we focused on the interactions between *Wolbachia* and reactive oxygen species, a central component of the arthropod immune response. In order to explain different effects of *Wolbachia* on the host oxidative environment, we proposed a hypothesis that emphasizes the importance of distinguishing between novel and coevolved *Wolbachia*–host associations when considering the mutual interactions between the symbiont and the host immune system. Building on findings of Chapter 4, we showed that a *Wolbachia*-induced immune response based on reactive oxygen species might be involved in antipathogenic effects, and perhaps even in *Wolbachia*-associated host protection. Lastly, we explored the possibility that some *Wolbachia* effects might be

attributable to mitohormesis, a process by which mild mitochondrial stress (i.e. slightly elevated levels of reactive oxygen species) improves systemic defense mechanisms (Chapter 6).

In summary, our findings suggest that both horizontal transmission and mutualistic effects contribute greatly to the *Wolbachia* pandemic among arthropods. Both issues are very up-to-date topics, as more and more papers appear that deal with either of them. Some of these publications seem to question the predominance of reproductive parasitism, either in favor of mutualistic effects (Hamm *et al.*, 2014) or of horizontal transmission (Parratt *et al.*, 2016). In this thesis, we prefer the view that it is the full range of *Wolbachia*'s abilities—vertical *and* horizontal transmission, reproductive parasitism *and* mutualistic effects—that explains their unparalleled success.

One important conclusion from our findings is that *Wolbachia* should be fully embraced as symbionts with mixed-mode transmission (if one takes different time scales into account). Although typically treated as a special case, mixed-mode transmission may in fact be common among symbionts and makes a big difference for their evolution (Ebert, 2013). We have shown here that this is particularly the case for *Wolbachia*. With the advent of more reliable estimates for the frequency of *Wolbachia* horizontal transmission and extinction events (Bailly-Bechet *et al.*, 2017), our modeling approach could serve as a starting point for future work to better understand the long-term spread and persistence of *Wolbachia*.

With respect to *Wolbachia*'s mutualistic effects, it is useful to relate them to the large body of literature on the evolution of mutualisms. Our current understanding of mutualisms is that they are best viewed as reciprocal exploitations that nonetheless provide net benefits to each partner (Doebeli and Knowlton, 1998; Herre *et al.*, 1999). From this point of view, mutualists are selected not to invariably maximize their partner's fitness (as classical thinking would have it), but rather to maximize their own net benefits (Sachs *et al.*, 2011a). As this thesis suggests, most *Wolbachia*–arthropod mutualisms seem to be adequately described by such an 'antagonistic arms race' framework for the evolution of mutualism (Sachs *et al.*, 2011a). This framework accommodates both the many instances of 'Jekyll and Hyde' infections and the fact that *Wolbachia* mutualisms frequently arise without selection to directly benefit hosts, that is, as byproduct benefits. Given that byproduct benefits have probably been involved in the evolutionary origin of many existing mutualisms (Connor, 1995; Bergstrom *et al.*, 2003), we may nevertheless expect the emergence of some 'real' *Wolbachia*–arthropod mutualisms. Future work will hopefully

reveal instances of such ultimate mutualisms. It will also be fruitful to elucidate how antagonistic arms races between *Wolbachia* and their hosts are affected by selection pressures from the host immune system, and how these selective pressures differ between more parasitic and more mutualistic interactions. Although there are some first efforts in this respect (Jiggins *et al.*, 2002b; Brownlie *et al.*, 2007), much remains to be done. In general, gaining a better insight into immunological aspects of *Wolbachia*–arthropod symbioses will be crucial if we are to fully understand the immense success of these bacteria.

Recent years have seen a growing interest in the interface between symbiosis, major transitions in evolution, and the nature of individuality and organismality (Queller and Strassmann, 2009; Sachs *et al.*, 2011b; Kiers and West, 2015; West *et al.*, 2015; Estrela *et al.*, 2016; Díaz-Muñoz *et al.*, 2016). One key issue relates to the question of what kinds of symbioses qualify as major evolutionary transitions, that is, as new organisms. Constitutive features of such major transitions are a high level of cooperation, low levels of conflict, and mutual dependence between partners. There is broad consensus that some obligate symbionts and their hosts meet the criteria to be considered organisms, e.g. *Buchnera* that provide their aphid hosts with essential nutrients and are housed in specialized host cells (bacteriocytes). Some *Wolbachia*–arthropod relationships come close to the *Buchnera*–aphid symbiosis: in the bedbug, for example, *Wolbachia* are bacteriocyte-associated obligate nutritional mutualists (Hosokawa *et al.*, 2010), suggesting that these symbioses, too, might be considered integrated organisms. The crucial difference, however, is that such highly integrated symbioses are rare among *Wolbachia*–arthropod associations, whereas they are the rule in *Buchnera*–aphid associations. In sum, therefore, the symbiosis between *Wolbachia* and arthropods does not constitute a major transition in evolution.

Prior to a major transition, the outcomes of species interactions are to a large extent context-dependent (Chamberlain *et al.*, 2014), and we have shown in this thesis that this also holds for *Wolbachia*–arthropod symbioses. Building on the organismality approach by Queller and Strassmann (2009) that defines an organism simply by having high cooperation and low conflict among its parts, Díaz-Muñoz *et al.* (2016) recently proposed the 'contextual organismality' framework. Within this framework, context-dependent cooperation is a stepping stone toward increased organismal integration, with organisms being defined by a lack of context dependence. We propose that the varying degrees of integration among *Wolbachia*–arthropod symbioses can be used to test the prediction that this variation will be

associated with different degrees of context dependence (Estrela *et al.*, 2016; Díaz-Muñoz *et al.*, 2016).

Finally, it might be worthwhile to relate our work to the currently popular 'hologenome concept' which also aims at a discussion of individuality and organismality (Zilber-Rosenberg and Rosenberg, 2008; Bordenstein and Theis, 2015). One of its major tenets is that the holobiont (the host and its microbiome), together with its hologenome, should be considered as the unit of selection, which requires concordance of selective interests between the host and its symbionts. However, even the selective interests of mutualistic *Wolbachia* almost never completely align with those of their hosts. Therefore, we agree with critics of the hologenome concept in that it is largely based on restrictive assumptions and only applies to a subset of symbiont–host interactions (Moran and Sloan, 2015; Douglas and Werren, 2016; Queller and Strassmann, 2016).

In closing, we hope to have convincingly argued for the involvement of horizontal transmission and mutualistic effects in the global *Wolbachia* pandemic among arthropods. The methods, findings, and reflections presented in this thesis are to stimulate future research on these fascinating symbionts and the creatures they live in. More than ever, biologists studying symbiosis might find themselves in a kind of 'future shock', in view of "too much change in too short a time" (McFall-Ngai, 2008).[1] In this spirit, we look forward with keen anticipation to the contributions and challenges *Wolbachia* will present to the field.

[1] In her essay, McFall-Ngai (2008) refers to the 1970 book *Future Shock* by Alvin Toffler, in which the author defines the term as "the shattering stress and disorientation that we induce in individuals by subjecting them to too much change in too short a time".

Appendix

A1 Infection dynamics with two strains, but without doubly infected hosts

In the first section of the appendix, we describe in detail how invasion conditions can be derived from the recursion equations, following an approach taken by Kriesner *et al.* (2013). The same approach is used in all further sections.

Two strains: CI

Invasion of a beneficial strain into a CI population

We consider a population to be infected with a CI strain at equilibrium frequency. We are interested in the conditions for an initially rare beneficial strain to increase in frequency. Individuals can be infected with a CI strain, with a beneficial strain, or uninfected. Thus we assume that individuals cannot be doubly infected with both strains. We denote the frequency of each female type by p_{CI}, $p_{\oplus}$, and p_{U}, respectively. The recursions for the frequencies of the three female types are

$$p'_{\mathrm{CI}} = \frac{(p_{\mathrm{CI}} F_{\mathrm{CI}} t_{\mathrm{CI}}) \left[p_{\mathrm{CI}} + p_{\oplus} + p_{\mathrm{U}}\right]}{\overline{w}}, \tag{A1a}$$

$$p'_{\oplus} = \frac{(p_{\oplus} F_{\oplus} t_{\oplus}) \left[(1 - l_{\mathrm{CI}})\, p_{\mathrm{CI}} + p_{\oplus} + p_{\mathrm{U}}\right]}{\overline{w}}, \tag{A1b}$$

$$\begin{aligned} p'_{\mathrm{U}} = {} & \left(p_{\mathrm{U}} + p_{\mathrm{CI}} F_{\mathrm{CI}} \left(1 - t_{\mathrm{CI}}\right) + p_{\oplus} F_{\oplus} \left(1 - t_{\oplus}\right)\right) \\ & \times \left[(1 - l_{\mathrm{CI}})\, p_{\mathrm{CI}} + p_{\oplus} + p_{\mathrm{U}}\right] \frac{1}{\overline{w}}, \end{aligned} \tag{A1c}$$

where

$$\begin{aligned} \overline{w} = 2\Big(& p_{\mathrm{CI}} F_{\mathrm{CI}} \left(\left(1 - l_{\mathrm{CI}} \left(1 - t_{\mathrm{CI}}\right)\right) p_{\mathrm{CI}} + p_{\oplus} + p_{\mathrm{U}}\right) + \\ & \left(p_{\oplus} F_{\oplus} + p_{\mathrm{U}}\right) \left(\left(1 - l_{\mathrm{CI}}\right) p_{\mathrm{CI}} + p_{\oplus} + p_{\mathrm{U}}\right) \Big). \end{aligned}$$

The first term in the numerator (in round brackets) denotes the maternal contribution, and the second term (in square brackets) denotes the paternal contribution. The frequencies of each type do not differ between the sexes, therefore the paternal contribution can be expressed in terms of female frequencies.

Now consider the condition for a beneficial strain to increase when it is extremely rare in a population infected at equilibrium with a CI strain. With $p_\oplus \approx 0$, this situation corresponds to the scenario described in section 5.3.1 (one-symbiont CI dynamics). Hence, the equilibrium frequency for the CI strain, $\hat{p}_{\mathrm{CI}}$, is given by equation (5.6a). When the CI strain is at equilibrium ($p'_{\mathrm{CI}} = p_{\mathrm{CI}} = \hat{p}_{\mathrm{CI}}$), we see from equation (A1a) that $\overline{w} = F_{\mathrm{CI}} t_{\mathrm{CI}} [p_{\mathrm{CI}} + p_\oplus + p_{\mathrm{U}}]$. The condition for the beneficial strain to increase when rare is $p'_\oplus > p_\oplus$ and thus, from equation (A1b), $F_\oplus t_\oplus [(1 - l_{\mathrm{CI}}) p_{\mathrm{CI}} + p_\oplus + p_{\mathrm{U}}] > \overline{w}$. Combining both equations, we get $F_\oplus t_\oplus [(1 - l_{\mathrm{CI}}) p_{\mathrm{CI}} + p_\oplus + p_{\mathrm{U}}] > F_{\mathrm{CI}} t_{\mathrm{CI}} [p_{\mathrm{CI}} + p_\oplus + p_{\mathrm{U}}]$. Considering that $p_{\mathrm{CI}} + p_\oplus + p_{\mathrm{U}} = 0.5$, we get $F_\oplus t_\oplus (0.5 - l_{\mathrm{CI}} \hat{p}_{\mathrm{CI}}) > F_{\mathrm{CI}} t_{\mathrm{CI}}/2$ and hence condition (5.11):

$$F_\oplus t_\oplus (1 - 2 l_{\mathrm{CI}} \hat{p}_{\mathrm{CI}}) > F_{\mathrm{CI}} t_{\mathrm{CI}}.$$

If the beneficial strain is able to rescue CI (i.e. if it is a $mod^- resc^+$ strain), equation (A1b) simplifies to $p'_\oplus = (p_\oplus F_\oplus t_\oplus) [p_{\mathrm{CI}} + p_\oplus + p_{\mathrm{U}}] / \overline{w}$. Hence, for the invasion condition for a beneficial $resc^+$ strain, we get condition (5.12):

$$F_\oplus t_\oplus > F_{\mathrm{CI}} t_{\mathrm{CI}}.$$

Two strains: MK

Invasion of a beneficial strain into a MK population

The recursions for the frequencies of the three female types are

$$p'_{\mathrm{MK}} = \frac{p_{\mathrm{MK}} R F_{\mathrm{MK}} t_{\mathrm{MK}}}{\overline{w}}, \tag{A2a}$$

$$p'_\oplus = \frac{p_\oplus F_\oplus t_\oplus}{\overline{w}}, \tag{A2b}$$

$$p'_{\mathrm{U}} = \frac{p_{\mathrm{U}} + p_{\mathrm{MK}} R F_{\mathrm{MK}} (1 - t_{\mathrm{MK}}) + p_\oplus F_\oplus (1 - t_\oplus)}{\overline{w}}, \tag{A2c}$$

where

$$\overline{w} = p_{\mathrm{MK}} R F_{\mathrm{MK}} (2 - (1 - v) t_{\mathrm{MK}}) + 2 (p_\oplus F_\oplus + p_{\mathrm{U}}).$$

Using the same reasoning as above, we derive at condition (5.14):

$$F_{\oplus}t_{\oplus} > RF_{\mathrm{MK}}t_{\mathrm{MK}}.$$

Two strains: CI and MK

Invasion of a MK strain into a CI population

The recursions for the frequencies of the three female types are

$$p'_{\mathrm{CI}} = \frac{(p_{\mathrm{CI}}F_{\mathrm{CI}}t_{\mathrm{CI}})\left[p_{\mathrm{CI}} + p_{\mathrm{MK}}v + p_{\mathrm{U}}\right]}{\overline{w}}, \tag{A3a}$$

$$p'_{\mathrm{MK}} = \frac{(p_{\mathrm{MK}}RF_{\mathrm{MK}}t_{\mathrm{MK}})\left[(1-l_{\mathrm{CI}})\,p_{\mathrm{CI}} + p_{\mathrm{MK}}v + p_{\mathrm{U}}\right]}{\overline{w}}, \tag{A3b}$$

$$\begin{aligned} p'_{\mathrm{U}} = {} & (p_{\mathrm{U}} + p_{\mathrm{CI}}F_{\mathrm{CI}}\,(1-t_{\mathrm{CI}}) + p_{\mathrm{MK}}RF_{\mathrm{MK}}\,(1-t_{\mathrm{MK}})) \\ & \times \left[(1-l_{\mathrm{CI}})\,p_{\mathrm{CI}} + p_{\mathrm{MK}}v + p_{\mathrm{U}}\right]\frac{1}{\overline{w}}, \end{aligned} \tag{A3c}$$

where

$$\begin{aligned} \overline{w} = {} & 2\,(p_{\mathrm{CI}}F_{\mathrm{CI}}t_{\mathrm{CI}})\left[p_{\mathrm{CI}} + p_{\mathrm{MK}}v + p_{\mathrm{U}}\right] + \\ & (1+v)\,(p_{\mathrm{MK}}RF_{\mathrm{MK}}t_{\mathrm{MK}})\left[(1-l_{\mathrm{CI}})\,p_{\mathrm{CI}} + p_{\mathrm{MK}}v + p_{\mathrm{U}}\right] \\ & +2\,(p_{\mathrm{U}} + p_{\mathrm{CI}}F_{\mathrm{CI}}\,(1-t_{\mathrm{CI}}) + p_{\mathrm{MK}}RF_{\mathrm{MK}}\,(1-t_{\mathrm{MK}})) \\ & \times \left[(1-l_{\mathrm{CI}})\,p_{\mathrm{CI}} + p_{\mathrm{MK}}v + p_{\mathrm{U}}\right]. \end{aligned}$$

Note that for the frequency of MK-infected males in the paternal contribution (in square brackets) we use the term $p_{\mathrm{MK}}v$ because it is the only male frequency that does not equal the female one. Using the same reasoning as above, we derive at condition (5.16):

$$RF_{\mathrm{MK}}t_{\mathrm{MK}}(1 - 2l_{\mathrm{CI}}\hat{p}_{\mathrm{CI}}) > F_{\mathrm{CI}}t_{\mathrm{CI}}.$$

A2 Infection dynamics with two strains and doubly infected hosts

The recursion equations for the frequencies of the four female types are:

$$\begin{aligned}
p'_{\mathrm{CI+MK}} &= \left(p_{\mathrm{CI+MK}} R F_{\mathrm{CI}} F_{\mathrm{MK}} t_{\mathrm{CI}} t_{\mathrm{MK}}\right) \\
&\quad \times \left[\left(p_{\mathrm{CI+MK}} + p_{\mathrm{MK}}\right) v + p_{\mathrm{CI}} + p_{\mathrm{U}}\right] \frac{1}{\overline{w}}, && \text{(A4a)} \\
p'_{\mathrm{CI}} &= \left(\left(p_{\mathrm{CI}} + p_{\mathrm{CI+MK}} R F_{\mathrm{MK}} \left(1 - t_{\mathrm{MK}}\right)\right) F_{\mathrm{CI}} t_{\mathrm{CI}}\right) \\
&\quad \times \left[\left(p_{\mathrm{CI+MK}} + p_{\mathrm{MK}}\right) v + p_{\mathrm{CI}} + p_{\mathrm{U}}\right] \frac{1}{\overline{w}}, && \text{(A4b)} \\
p'_{\mathrm{MK}} &= \left(\left(p_{\mathrm{MK}} + p_{\mathrm{CI+MK}} F_{\mathrm{CI}} \left(1 - t_{\mathrm{CI}}\right)\right) R F_{\mathrm{MK}} t_{\mathrm{MK}}\right) \\
&\quad \times \left[\left(1 - l_{\mathrm{CI}}\right) \left(p_{\mathrm{CI+MK}} v + p_{\mathrm{CI}}\right) + p_{\mathrm{MK}} v + p_{\mathrm{U}}\right] \frac{1}{\overline{w}}, && \text{(A4c)} \\
p'_{\mathrm{U}} &= \Big(p_{\mathrm{U}} + p_{\mathrm{CI+MK}} R F_{\mathrm{CI}} F_{\mathrm{MK}} \left(1 - t_{\mathrm{CI}}\right) \left(1 - t_{\mathrm{MK}}\right) + \\
&\quad p_{\mathrm{CI}} F_{\mathrm{CI}} \left(1 - t_{\mathrm{CI}}\right) + p_{\mathrm{MK}} R F_{\mathrm{MK}} \left(1 - t_{\mathrm{MK}}\right)\Big) \\
&\quad \times \left[\left(1 - l_{\mathrm{CI}}\right) \left(p_{\mathrm{CI+MK}} v + p_{\mathrm{CI}}\right) + p_{\mathrm{MK}} v + p_{\mathrm{U}}\right] \frac{1}{\overline{w}}, && \text{(A4d)}
\end{aligned}$$

where

$$\begin{aligned}
\overline{w} = {} & \left(1 + v\right) \left(p_{\mathrm{CI+MK}} R F_{\mathrm{CI}} F_{\mathrm{MK}} t_{\mathrm{CI}} t_{\mathrm{MK}}\right) \left[\left(p_{\mathrm{CI+MK}} + p_{\mathrm{MK}}\right) v + p_{\mathrm{CI}} + p_{\mathrm{U}}\right] \\
& + 2 \left(\left(p_{\mathrm{CI}} + p_{\mathrm{CI+MK}} R F_{\mathrm{MK}} \left(1 - t_{\mathrm{MK}}\right)\right) F_{\mathrm{CI}} t_{\mathrm{CI}}\right) \\
& \times \left[\left(p_{\mathrm{CI+MK}} + p_{\mathrm{MK}}\right) v + p_{\mathrm{CI}} + p_{\mathrm{U}}\right] \\
& + \left(1 + v\right) \left(\left(p_{\mathrm{MK}} + p_{\mathrm{CI+MK}} F_{\mathrm{CI}} \left(1 - t_{\mathrm{CI}}\right)\right) R F_{\mathrm{MK}} t_{\mathrm{MK}}\right) \\
& \times \left[\left(1 - l_{\mathrm{CI}}\right) \left(p_{\mathrm{CI+MK}} v + p_{\mathrm{CI}}\right) + p_{\mathrm{MK}} v + p_{\mathrm{U}}\right] \\
& + 2 \Big(p_{\mathrm{U}} + p_{\mathrm{CI+MK}} R F_{\mathrm{CI}} F_{\mathrm{MK}} \left(1 - t_{\mathrm{CI}}\right) \left(1 - t_{\mathrm{MK}}\right) + p_{\mathrm{CI}} F_{\mathrm{CI}} \left(1 - t_{\mathrm{CI}}\right) \\
& + p_{\mathrm{MK}} R F_{\mathrm{MK}} \left(1 - t_{\mathrm{MK}}\right)\Big) \\
& \times \left[\left(1 - l_{\mathrm{CI}}\right) \left(p_{\mathrm{CI+MK}} v + p_{\mathrm{CI}}\right) + p_{\mathrm{MK}} v + p_{\mathrm{U}}\right].
\end{aligned}$$

Bibliography

Aanen, D. K. and R. F. Hoekstra, 2007 The evolution of obligate mutualism: if you can't beat 'em, join 'em. Trends Ecol. Evol. **22**: 506–509.

Ackermann, M., R. Bijlsma, A. C. James, L. Partridge, B. J. Zwaan, and S. C. Stearns, 2001 Effects of assay conditions in life history experiments with *Drosophila melanogaster*. J. Evol. Biol. **14**: 199–209.

Aguirre, J. and J. D. Lambeth, 2010 Nox enzymes from fungus to fly to fish and what they tell us about Nox function in mammals. Free Radic. Biol. Med. **49**: 1342–1353.

Ahmed, M. Z., J. W. Breinholt, and A. Y. Kawahara, 2016 Evidence for common horizontal transmission of *Wolbachia* among butterflies and moths. BMC Evol. Biol. **16**: 118.

Ahmed, M. Z., O. F. C. Greyvenstein, C. Erasmus, J. J. Welch, and J. M. Greeff, 2013 Consistently high incidence of *Wolbachia* in global fig wasp communities. Ecol. Entomol. **38**: 147–154.

Ahmed, M. Z., S.-J. Li, X. Xue, X.-J. Yin, S.-X. Ren, F. M. Jiggins, *et al.*, 2015 The intracellular bacterium *Wolbachia* uses parasitoid wasps as phoretic vectors for efficient horizontal transmission. PLoS Pathog. **11**: e1004672.

Albertson, R., C. Casper-Lindley, J. Cao, U. Tram, and W. Sullivan, 2009 Symmetric and asymmetric mitotic segregation patterns influence *Wolbachia* distribution in host somatic tissue. J. Cell Sci. **122**: 4570–4583.

Alexandrov, I. D., M. V. Alexandrova, I. I. Goryacheva, N. V. Rochina, E. V. Shaikevich, and I. A. Zakharov, 2007 Removing endosymbiotic *Wolbachia* specifically decreases lifespan of females and competitiveness in a laboratory strain of *Drosophila melanogaster*. Russ. J. Genet. **43**: 1147–1152.

Aliota, M. T., S. A. Peinado, I. D. Velez, and J. E. Osorio, 2016 The *w*Mel strain of *Wolbachia* reduces transmission of Zika virus by *Aedes aegypti*. Sci. Rep. **6**: 28792.

Almeida, F., A. S. Moura, A. F. Cardoso, C. E. Winter, A. T. Bijovsky, and L. Suesdek, 2011 Effects of *Wolbachia* on fitness of *Culex quinquefasciatus* (Diptera; Culicidae). Infect. Genet. Evol. **11**: 2138–2143.

Andersen, S. B., M. Boye, D. R. Nash, and J. J. Boomsma, 2012 Dynamic *Wolbachia* prevalence in *Acromyrmex* leaf-cutting ants: potential for a nutritional symbiosis. J. Evol. Biol. **25**: 1340–1350.

Anderson, R. M. and R. M. May, 1982 Coevolution of hosts and parasites. Parasitology **85**: 411–426.

Andrews, E. S., P. R. Crain, Y. Fu, D. K. Howe, and S. L. Dobson, 2012 Reactive oxygen species production and *Brugia pahangi* survivorship in *Aedes polynesiensis* with artificial *Wolbachia* infection types. PLoS Pathogens **8**: e1003075.

Arakaki, N., T. Miyoshi, and H. Noda, 2001a Parthenogenesis induced by *Wolbachia* in *Gronotoma micromorpha* (Hymenoptera: Eucolidae). Entomol. Sci. **4**: 9–15.

Arakaki, N., T. Miyoshi, and H. Noda, 2001b *Wolbachia*-mediated parthenogenesis in the predatory thrips *Franklinothrips vespiformis* (Thysanoptera: Insecta). Proc. R. Soc. Lond. B **268**: 1011–1016.

Arakaki, N., H. Noda, and K. Yamagishi, 2000 *Wolbachia*-induced parthenogenesis in the egg parasitoid *Telenomus nawai*. Entomol. Exp. Appl. **96**: 177–184.

Arthofer, W., M. Riegler, D. N. Avtzis, and C. Stauffer, 2009a Evidence for low-titre infections in insect symbiosis: *Wolbachia* in the bark beetle *Pityogenes chalcographus* (Coleoptera, Scolytinae). Environ. Microbiol. **11**: 1923–1933.

Arthofer, W., M. Riegler, D. Schneider, M. Krammer, W. J. Miller, and C. Stauffer, 2009b Hidden *Wolbachia* diversity in field populations of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera, Tephritidae). Mol. Ecol. **18**: 3816–3830.

Asrat, S., D. A. de Jesús, A. D. Hempstead, V. Ramabhadran, and R. R. Isberg, 2014 Bacterial pathogen manipulation of host membrane trafficking. Annu. Rev. Cell Dev. Biol. **30**: 79–109.

Atyame, C. M., F. Delsuc, N. Pasteur, M. Weill, and O. Duron, 2011 Diversification of *Wolbachia* endosymbiont in the *Culex pipiens* mosquito. Mol. Biol. Evol. **28**: 2761–2772.

Bae, Y. S., M. K. Choi, and W.-J. Lee, 2010 Dual oxidase in mucosal immunity and host–microbe homeostasis. Trends Immunol. **31**: 278–287.

Bailly-Bechet, M., P. Martins-Simões, G. Szöllősi, G. Mialdea, M.-F. Sagot, and S. Charlat, 2017 How long does *Wolbachia* remain on board? Mol. Biol. Evol. p. doi:10.1093/molbev/msx073.

Balaban, R. S., S. Nemoto, and T. Finkel, 2005 Mitochondria, oxidants, and aging. Cell **120**: 483–495.

Balas, M. T., M. H. Lee, and J. H. Werren, 1996 Distribution and fitness effects of the son-killer bacterium in *Nasonia*. Evol. Ecol. **10**: 593–607.

Baldini, F., N. Segata, J. Pompon, P. Marcenac, W. R. Shaw, R. K. Dabiré, A. Diabaté, *et al.*, 2014 Evidence of natural *Wolbachia* infections in field populations of *Anopheles gambiae*. Nat. Commun. **5**: 3985.

Baldo, L., N. A. Ayoub, C. Y. Hayashi, J. A. Russell, J. K. Stahlhut, and J. H. Werren, 2008 Insight into the routes of *Wolbachia* invasion: high levels of horizontal transfer in the spider genus *Agelenopsis* revealed by *Wolbachia* strain and mitochondrial DNA diversity. Mol. Ecol. **17**: 557–569.

Baldo, L., J. C. Dunning Hotopp, K. A. Jolley, S. R. Bordenstein, S. A. Biber, R. R. Choudhury, C. Hayashi, M. C. J. Maiden, H. Tettelin, and J. H. Werren, 2006 Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Appl. Environ. Microbiol. **72**: 7098–7110.

Ballard, J. W. O., 2005 *Drosophila simulans* as a novel model for studying mitochondrial metabolism and aging. Exp. Gerontol. **40**: 763–773.

Ballard, J. W. O. and R. G. Melvin, 2007 Tetracycline treatment influences mitochondrial metabolism and mtDNA density two generations after treatment in *Drosophila*. Insect Mol. Biol. **16**: 799–802.

Baltanás, A., M. Zabal-Aguirre, M. Pita, and C. López-Fernández, 2007 *Wolbachia* identified in a new crustacean host: an explanation of the prevalence of asexual reproduction in non-marine ostracods? Fundam. Appl. Limnol. **169**: 217–221.

Barr, K. L., L. B. Hearne, S. Briesacher, T. L. Clark, and G. E. Davis, 2010 Microbial symbionts in insects influence down-regulation of defense genes in maize. PLoS ONE **5**: e11339.

Bass, B. P., K. Cullen, and K. McCall, 2007 The axon guidance gene *lola* is required for programmed cell death in the *Drosophila* ovary. Dev. Biol. **304**: 771–785.

Baton, L. A., E. C. Pacidônio, D. d. S. Gonçalves, and L. A. Moreira, 2013 *w*Flu: Characterization and evaluation of a native *Wolbachia* from the mosquito *Aedes fluviatilis* as a potential vector control agent. PLoS ONE **8**: e59619.

Bazzocchi, C., S. Comazzi, R. Santoni, C. Bandi, C. Genchi, and M. Mortarino, 2007 *Wolbachia* surface protein (WSP) inhibits apoptosis in human neutrophils. Parasite Immunol. **29**: 73–79.

Bechsgaard, J., B. Vanthournout, P. Funch, S. Vestbo, R. A. Gibbs, S. Richards, K. W. Sanggaard, *et al.*, 2016 Comparative genomic study of arachnid immune systems indicates loss of beta-1,3-glucanase-related proteins and the immune deficiency pathway. J. Evol. Biol. **29**: 277–291.

Beckmann, J. F. and A. M. Fallon, 2013 Detection of the *Wolbachia* protein WPIP0282 in mosquito spermathecae: implications for cytoplasmic incompatibility. Insect Biochem. Mol. Biol. **43**: 867–878.

Beckmann, J. F., J. A. Ronau, and M. Hochstrasser, 2017 A *Wolbachia* deubiquitylating enzyme induces cytoplasmic incompatibility. Nat. Microbiol. **2**: 17007.

Bergstrom, C. T., J. L. Bronstein, R. Bshary, R. C. Connor, M. Daly, S. A. Frank, H. Gintis, *et al.*, 2003 Group report: interspecific mutualism—puzzles and predictions. In *Genetic and Cultural*

Evolution of Cooperation, edited by P. Hammerstein, pp. 241–256, MIT Press, Cambridge, MA.

Bian, G., D. Joshi, Y. Dong, P. Lu, G. Zhou, X. Pan, Y. Xu, G. Dimopoulos, and Z. Xi, 2013 *Wolbachia* invades *Anopheles stephensi* populations and induces refractoriness to *Plasmodium* infection. Science **340**: 748–751.

Bian, G., Y. Xu, P. Lu, Y. Xie, and Z. Xi, 2010 The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. PLoS Pathogens **6**: e1000833.

Blagrove, M. S. C., C. Arias-Goeta, C. Di Genua, A.-B. Failloux, and S. P. Sinkins, 2013 A *Wolbachia w*Mel transinfection in *Aedes albopictus* is not detrimental to host fitness and inhibits Chikungunya virus. PLoS Negl. Trop. Dis. **7**: e2152.

Blagrove, M. S. C., C. Arias-Goeta, A.-B. Failloux, and S. P. Sinkins, 2012 *Wolbachia* strain wMel induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus*. Proc. Natl. Acad. Sci. U.S.A. **109**: 255–260.

Bonnay, F., E. Cohen-Berros, M. Hoffmann, S. Y. Kim, G. L. Boulianne, J. A. Hoffmann, *et al.*, 2013 *big bang* gene modulates gut immune tolerance in *Drosophila*. Proc. Natl. Acad. Sci. U.S.A. **110**: 2957–2962.

Bordenstein, S. R. and K. R. Theis, 2015 Host biology in light of the microbiome: ten principles of holobionts and hologenomes. PLoS Biol. **13**: e1002226.

Bordenstein, S. R. and J. H. Werren, 2000 Do *Wolbachia* influence fecundity in *Nasonia vitripennis*? Heredity **84**: 54–62.

Bosco-Drayon, V., M. Poidevin, I. G. Boneca, K. Narbonne-Reveau, J. Royet, and B. Charroux, 2012 Peptidoglycan sensing by the receptor PGRP-LE in the *Drosophila* gut induces immune responses to infectious bacteria and tolerance to microbiota. Cell Host Microbe **12**: 153–165.

Bossan, B., A. Koehncke, and P. Hammerstein, 2011 A new model and method for understanding *Wolbachia*-induced cytoplasmic incompatibility. PLoS ONE **6**: e19757.

Bouchon, D., R. Cordaux, and P. Grève, 2008 Feminizing *Wolbachia* and the evolution of sex determination in isopods. In *Insect Symbiosis. Volume 3*, edited by K. Bourtzis and T. A. Miller, pp. 273–294, CRC Press, Boca Raton.

Bourtzis, K., M. M. Pettigrew, and S. L. O'Neill, 2000 *Wolbachia* neither induces nor suppresses transcripts encoding antimicrobial peptides. Insect Mol. Biol. **9**: 635–639.

Bouwma, A. M. and D. Shoemaker, 2011 *Wolbachia* wSinvictaA infections in natural populations of the fire ant *Solenopsis invicta*: Testing for phenotypic effects. J. Insect Sci. **11**: 11.

Braquart-Varnier, C., M. Raimond, G. Mappa, F. D. Chevalier, W. Le Clec'h, and M. Sicard, 2015 The hematopoietic organ: a cornerstone for *Wolbachia* propagation between and within hosts. Front. Microbiol. **6**: 1424.

Breeuwer, J. A. J. and J. H. Werren, 1990 Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. Nature **346**: 558–560.

Brelsfoard, C. L. and S. L. Dobson, 2011 *Wolbachia* effects on host fitness and the influence of male aging on cytoplasmic incompatibility in *Aedes polynesiensis* (Diptera: Culicidae). J. Med. Entomol. **48**: 1008–1015.

Brennan, L. J., J. A. Haukedal, J. C. Earle, B. Keddie, and H. L. Harris, 2012 Disruption of redox homeostasis leads to oxidative DNA damage in spermatocytes of *Wolbachia*-infected *Drosophila simulans*. Insect Mol. Biol. **21**: 510–520.

Brennan, L. J., B. A. Keddie, H. R. Braig, and H. L. Harris, 2008 The endosymbiont *Wolbachia pipientis* induces the expression of host antioxidant proteins in an *Aedes albopictus* cell line. PLoS ONE **3**: e2083.

Bronstein, J. L., 1994 Conditional outcomes in mutualistic interactions. Trends Ecol. Evol. **9**: 214–217.

Brown, A. M. V., S. K. Wasala, D. K. Howe, A. B. Peetz, I. A. Zasada, and D. Denver, 2016 Genomic evidence for plant-parasitic nematodes as the earliest *Wolbachia* hosts. Sci. Rep. **6**: 34955.

Brown, A. N. and V. K. Lloyd, 2015 Evidence for horizontal transfer of *Wolbachia* by a *Drosophila* mite. Exp. Appl. Acarol. **66**: 301–311.

Brownlie, J. C., M. Adamski, B. Slatko, and E. A. McGraw, 2007 Diversifying selection and host adaptation in two endosymbiont genomes. BMC Evol. Biol. **7**: 68.

Brownlie, J. C., B. N. Cass, M. Riegler, J. J. Witsenburg, I. Iturbe-Ormaetxe, E. A. McGraw, and S. L. O'Neill, 2009 Evidence for metabolic provisioning by a common invertebrate endosymbiont, *Wolbachia pipientis*, during periods of nutritional stress. PLoS Pathog. **5**: e1000368.

Brownlie, J. C. and K. N. Johnson, 2009 Symbiont-mediated protection in insect hosts. Trends Microbiol. **17**: 348–354.

Brucker, R. M. and S. R. Bordenstein, 2012 Speciation by symbiosis. Trends Ecol. Evol. **27**: 443–451.

Buchon, N., N. Silverman, and S. Cherry, 2014 Immunity in *Drosophila melanogaster*–from microbial recognition to whole-organism physiology. Nat. Rev. Immunol. **14**: 796–810.

Burt, A. and R. Trivers, 2006 *Genes in Conflict. The Biology of Selfish Genetic Elements*. Belknap Press, Cambridge, MA.

Callaini, G., R. Dallai, and M. G. Riparbelli, 1997 *Wolbachia*-induced delay of paternal chromatin condensation does not prevent maternal chromosomes from entering anaphase in incompatible crosses of *Drosophila simulans*. J. Cell Sci. **110**: 271–280.

Callaini, G., M. G. Riparbelli, and R. Dallai, 1994 The distribution of cytoplasmic bacteria in the early *Drosophila* embryo is mediated by astral microtubules. J. Cell Sci. **107**: 673–682.

Callaway, E., 2016 Infected mosquitoes fight Zika. Nature **539**: 17–18.

Caragata, E. P., H. L. C. Dutra, and L. A. Moreira, 2016 Exploiting intimate relationships: controlling mosquito-transmitted disease with *Wolbachia*. Trends Parasitol. **32**: 207–218.

Caragata, E. P., E. Rancès, L. M. Hedges, A. W. Gofton, K. N. Johnson, S. L. O'Neill, and E. A. McGraw, 2013 Dietary cholesterol modulates pathogen blocking by *Wolbachia*. PLoS Pathog. **9**: e1003459.

Carrondo, M. A., 2003 Ferritins, iron uptake and storage from the bacterioferritin viewpoint. EMBO J. **22**: 1959–1968.

Carson, H. L., L. S. Chang, and T. W. Lyttle, 1982 Decay of female sexual behavior under parthenogenesis. Science **218**: 68–70.

Caspari, E. and G. S. Watson, 1959 On the evolutionary importance of cytoplasmic sterility in mosquitoes. Evolution **13**: 568–570.

Cassat, J. E. and E. P. Skaar, 2013 Iron in infection and immunity. Cell Host Microbe **13**: 509–519.

Chakrabarti, S., M. Poidevin, and B. Lemaitre, 2014 The *Drosophila* MAPK p38c regulates oxidative stress and lipid homeostasis in the intestine. PLoS Genet. **10**: e1004659.

Chamberlain, S. A., J. L. Bronstein, and J. A. Rudgers, 2014 How context dependent are species interactions? Ecol. Lett. **17**: 881–890.

Charlat, S., J. Engelstädter, E. A. Dyson, E. A. Hornett, A. Duplouy, P. Tortosa, N. Davies, G. K. Roderick, N. Wedell, and G. D. D. Hurst, 2006 Competing selfish genetic elements in the butterfly *Hypolimnas bolina*. Curr. Biol. **16**: 2453–2458.

Charlat, S., E. A. Hornett, E. A. Dyson, P. P. Y. Ho, N. T. Loc, M. Schilthuizen, N. Davies, G. K. Roderick, and G. D. D. Hurst, 2005 Prevalence and penetrance variation of male-killing *Wolbachia* across Indo-Pacific populations of the butterfly *Hypolimnas bolina*. Mol. Ecol. **14**: 3525–3530.

Charlat, S., E. A. Hornett, J. H. Fullard, N. Davies, G. K. Roderick, N. Wedell, and G. D. Hurst, 2007 Extraordinary flux in sex ratio. Science **317**: 214.

Charlat, S., L. Le Chat, and H. Merçot, 2003 Characterization of non-cytoplasmic incompatibility inducing *Wolbachia* in two continental African populations of *Drosophila simulans*. Heredity **90**: 49–55.

Chávez, V., A. Mohri-Shiomi, A. Maadani, L. A. Vega, and D. A. Garsin, 2007 Oxidative stress enzymes are required for DAF-16-mediated immunity due to generation of reactive oxygen species by *Caenorhabditis elegans*. Genetics **176**: 1567–1577.

Chen, S.-J., F. Lu, J.-A. Cheng, M.-X. Jiang, and M. O. Way, 2012 Identification and biological role of the endosymbionts *Wolbachia* in rice water weevil (Coleoptera: Curculionidae). Environ. Entomol. **41**: 469–477.

Cheng, G., Y. Liu, P. Wang, and X. Xiao, 2016 Mosquito defense strategies against viral infection. Trends Parasitol. **32**: 177–186.

Cheng, Q., T. D. Ruel, W. Zhou, S. K. Moloo, P. Majiwa, S. L. O'Neill, *et al.*, 2000 Tissue distribution and prevalence of *Wolbachia* infections in tsetse flies, *Glossina* spp. Med. Vet. Entomol. **14**: 44–50.

Chevalier, F., J. Herbinière-Gaboreau, D. Charif, G. Mitta, F. Gavory, P. Wincker, *et al.*, 2012 Feminizing *Wolbachia*: a transcriptomics approach with insights on the immune response genes in *Armadillidium vulgare*. BMC Microbiol. **12**: S1.

Chiel, E., E. Zchori-Fein, M. Inbar, Y. Gottlieb, T. Adachi-Hagimori, S. E. Kelly, M. K. Asplen, and M. S. Hunter, 2009 Almost there: transmission routes of bacterial symbionts between trophic levels. PLoS ONE **4**: e4767.

Cho, K.-O., G.-W. Kim, and O.-K. Lee, 2011 *Wolbachia* bacteria reside in host Golgi-related vesicles whose position is regulated by polarity proteins. PLoS ONE **6**: e22703.

Choy, A. and C. R. Roy, 2013 Autophagy and bacterial infection: an evolving arms race. Trends Microbiol. **21**: 451–456.

Chrostek, E., M. S. P. Marialva, S. S. Esteves, L. A. Weinert, J. Martinez, F. M. Jiggins, and L. Teixeira, 2013 *Wolbachia* variants induce differential protection to viruses in *Drosophila melanogaster*: a phenotypic and phylogenomic analysis. PLoS Genet. **9**: e1003896.

Chrostek, E., M. S. P. Marialva, R. Yamada, S. L. O'Neill, and L. Teixeira, 2014 High anti-viral protection without immune upregulation after interspecies *Wolbachia* transfer. PLoS ONE **9**: e99025.

Clancy, D. J., D. Gems, L. G. Harshman, S. Oldham, H. Stocker, E. Hafen, S. J. Leevers, and L. Partridge, 2001 Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. Science **292**: 104–106.

Clancy, D. J. and A. A. Hoffmann, 1997 Behavior of *Wolbachia* endosymbionts from *Drosophila simulans* in *Drosophila serrata*, a novel host. Am. Nat. **149**: 975–988.

Clark, M. E., C. L. Anderson, J. Cande, and T. L. Karr, 2005 Widespread prevalence of *Wolbachia* in laboratory stocks and the implications for *Drosophila* research. Genetics **170**: 1667–1675.

Clarke, T. E. and R. J. Clem, 2003 Insect defenses against virus infection: the role of apoptosis. Int. Rev. Immunol. **22**: 401–424.

Comandatore, F., D. Sassera, M. Montagna, S. Kumar, G. Koutsovoulos, G. Thomas, C. Repton, *et al.*, 2013 Phylogenomics and analysis of shared genes suggest a single transition to mutualism in *Wolbachia* of nematodes. Genome Biol. Evol. **5**: 1668–1674.

Connor, R. C., 1995 The benefits of mutualism: a conceptual framework. Biol. Rev. **70**: 427–457.

Cook, P. E. and E. A. McGraw, 2010 *Wolbachia pipientis*: an expanding bag of tricks to explore for disease control. Trends Parasitol. **26**: 373–375.

Cordaux, R., D. Bouchon, and P. Grève, 2011 The impact of endosymbionts on the evolution of host sex-determination mechanisms. Trends Genet. **27**: 332–341.

Cordaux, R., A. Michel-Salzat, and D. Bouchon, 2001 *Wolbachia* infection in crustaceans: novel hosts and potential routes for horizontal transmission. J. Evol. Biol. **14**: 237–243.

Cordaux, R., S. Pichon, H. B. A. Hatira, V. Doublet, P. Grève, I. Marcadé, C. Braquart-Varnier, *et al.*, 2012 Widespread *Wolbachia* infection in terrestrial isopods and other crustaceans. ZooKeys **176**: 123–131.

Cosmides, L. M. and J. Tooby, 1981 Cytoplasmic inheritance and intragenomic conflict. J. Theor. Biol. **89**: 83–129.

Covacin, C. and S. C. Barker, 2007 Supergroup F *Wolbachia* bacteria parasitise lice (Insecta: Phthiraptera). Parasitol. Res. **100**: 479–485.

Curry, M. M., L. V. Paliulis, K. D. Welch, J. D. Harwood, and J. A. White, 2015 Multiple endosymbiont infections and reproductive manipulations in a linyphiid spider population. Heredity **115**: 146–152.

De Barro, P. J. and P. J. Hart, 2001 Antibiotic curing of parthenogenesis in *Eretmocerus mundus* (Australian parthenogenic form). Entomol. Exp. Appl. **99**: 225–230.

De Bary, A., 1879 *Die Erscheinung der Symbiose*. Karl J. Trubner, Strasbourg.

De Haes, W., L. Frooninckx, R. Van Assche, A. Smolders, G. Depuydt, J. Billen, *et al.*, 2014 Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. Proc. Natl. Acad. Sci. U.S.A. **111**: E2501–E2509.

De Mazancourt, C., M. Loreau, and U. Dieckmann, 2005 Understanding mutualism when there is adaptation to the partner. J. Ecol. **93**: 305–314.

Dedeine, F., M. Ahrens, L. Calcaterra, and D. D. Shoemaker, 2005a Social parasitism in fire ants (*Solenopsis* spp.): a potential mechanism for interspecies transfer of *Wolbachia*. Mol. Ecol. **14**: 1543–1548.

Dedeine, F., C. Bandi, M. Boulétreau, and L. H. Kramer, 2003 Insights into *Wolbachia* obligatory symbiosis. In *Insect Symbiosis*, edited by K. Bourtzis and T. A. Miller, pp. 267–282, CRC Press, Boca Raton.

Dedeine, F., M. Boulétreau, and F. Vavre, 2005b *Wolbachia* requirement for oogenesis: occurrence within the genus *Asobara* (Hymenoptera, Braconidae) and evidence for intraspecific variation in *A. tabida*. Heredity **95**: 394–400.

Dedeine, F., F. Vavre, F. Fleury, B. Loppin, M. E. Hochberg, and M. Boulétreau, 2001 Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp. Proc. Natl. Acad. Sci. U.S.A. **98**: 6247–6252.

Dedeine, F., F. Vavre, D. D. Shoemaker, and M. Boulétreau, 2004 Intra-individual coexistence of a *Wolbachia* strain required for host oogenesis with two strains inducing cytoplasmic incompatibility in the wasp *Asobara tabida*. Evolution **58**: 2167–2174.

Deretic, V., 2010 Autophagy in infection. Curr. Opin. Cell Biol. **22**: 252–262.

Díaz-Muñoz, S. L., A. M. Boddy, G. Dantas, C. M. Waters, and J. L. Bronstein, 2016 Contextual organismality: Beyond pattern to process in the emergence of organisms. Evolution **70**: 2669–2677.

Dixon, S. J. and B. R. Stockwell, 2014 The role of iron and reactive oxygen species in cell death. Nature Chem. Biol. **10**: 9–17.

Dobson, S. L., K. Bourtzis, H. R. Braig, B. F. Jones, W. Zhou, F. Rousset, *et al.*, 1999 *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. Insect Biochem. Mol. Biol. **29**: 153–160.

Dobson, S. L., E. J. Marsland, and W. Rattanadechakul, 2002 Mutualistic *Wolbachia* infection in *Aedes albopictus*: accelerating cytoplasmic drive. Genetics **160**: 1087–1094.

Dobson, S. L., W. Rattanadechakul, and E. J. Marsland, 2004 Fitness advantage and cytoplasmic incompatibility in *Wolbachia* single- and superinfected *Aedes albopictus*. Heredity **93**: 135–142.

Doebeli, M. and N. Knowlton, 1998 The evolution of interspecific mutualisms. Proc. Natl. Acad. Sci. U.S.A. **95**: 8676–8680.

Dong, P., J. J. Wang, F. Hu, and F. X. Jia, 2007 Influence of *Wolbachia* infection on the fitness of the stored-product pest *Liposcelis tricolor* (Psocoptera: Liposcelididae). J. Econ. Entomol. **100**: 1476–1481.

Dostert, C., E. Jouanguy, P. Irving, L. Troxler, D. Galiana-Arnoux, C. Hetru, J. A. Hoffmann, and J.-L. Imler, 2005 The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of *Drosophila*. Nat. Immunol. **6**: 946–953.

Doudoumis, V., G. Tsiamis, F. Wamwiri, C. Brelsfoard, U. Alam, E. Aksoy, S. Dalaperas, A. Abd-Alla, J. Ouma, P. Takac, S. Aksoy, and K. Bourtzis, 2012 Detection and characterization of *Wolbachia* infections in laboratory and natural populations of different species of tsetse flies (genus *Glossina*). BMC Microbiol. **12** (Suppl. 1): S3.

Douglas, A. E., 2010 *The Symbiotic Habit*. Princeton University Press, Princeton.

Douglas, A. E. and D. C. Smith, 1989 Are endosymbioses mutualistic? Trends Ecol. Evol. **4**: 350–352.

Douglas, A. E. and J. H. Werren, 2016 Holes in the hologenome: why host–microbe symbioses are not holobionts. mBio **7**: e02099–15.

Dowling, D. K. and L. W. Simmons, 2009 Reactive oxygen species as universal constraints in life-history evolution. Proc. R. Soc. B **276**: 1737–1745.

Driver, C., A. Georgiou, and G. Georgiou, 2004 The contribution by mitochondrially induced oxidative damage to aging in *Drosophila melanogaster*. Biogerontology **5**: 185–192.

Dunning Hotopp, J. C., M. Lin, R. Madupu, J. Crabtree, S. V. Angiuoli, J. Eisen, *et al.*, 2006 Comparative genomics of emerging human ehrlichiosis agents. PLoS Genet. **2**: e21.

Duplouy, A., G. D. D. Hurst, S. L. O'Neill, and S. Charlat, 2010 Rapid spread of male-killing *Wolbachia* in the butterfly *Hypolimnas bolina*. J. Evol. Biol. **23**: 231–235.

Duron, O., D. Bouchon, S. Boutin, L. Bellamy, L. Zhou, J. Engelstädter, and G. D. Hurst, 2008 The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. BMC Biol. **6**: 27.

Duron, O., T. E. Wilkes, and G. D. D. Hurst, 2010 Interspecific transmission of a male-killing bacterium on an ecological timescale. Ecol. Lett. **13**: 1139–1148.

Düsing, K., 1883 Die Factoren, welche die Sexualität entscheiden. Jenaische Zeitschrift für Naturwissenschaft **16**: 428–464.

Dutra, H. L. C., M. N. Rocha, F. B. S. Dias, S. B. Mansur, E. P. Caragata, and L. A. Moreira, 2016 *Wolbachia* blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. Cell Host Microbe **19**: 771–774.

Dutton, T. J. and S. P. Sinkins, 2005 Filarial susceptibility and effects of *Wolbachia* in *Aedes pseudoscutellaris* mosquitoes. Med. Vet. Entomol. **19**: 60–65.

Dyer, K. A. and J. Jaenike, 2004 Evolutionarily stable infection by a male-killing endosymbiont in *Drosophila innubila*: molecular evidence from the host and parasite genomes. Genetics **168**: 1443–1455.

Ebert, D., 2013 The epidemiology and evolution of symbionts with mixed-mode transmission. Annu. Rev. Ecol. Evol. Syst. **44**: 623–643.

Edwards, D. P., 2009 The roles of tolerance in the evolution, maintenance and breakdown of mutualism. Naturwissenschaften **96**: 1137–1145.

Egas, M., F. Vala, and J. A. J. Breeuwer, 2002 On the evolution of cytoplasmic incompatibility in haplodiploid species. Evolution **56**: 1101–1109.

Eleftherianos, I., J. Atri, J. Accetta, and J. C. Castillo, 2013 Endosymbiotic bacteria in insects: guardians of the immune system? Front. Physiol. **4**: 46.

Ellers, J., E. T. Kiers, C. R. Currie, B. R. McDonald, and B. Visser, 2012 Ecological interactions drive evolutionary loss of traits. Ecol. Lett. **15**: 1071–1082.

Elnagdy, S., M. E. N. Majerus, and L.-J. Lawson Handley, 2011 The value of an egg: resource reallocation in ladybirds (Coleoptera: Coccinellidae) infected with male-killing bacteria. J. Evol. Biol. **24**: 2164–2172.

Engelstädter, J. and G. D. Hurst, 2009 The ecology and evolution of microbes that manipulate host reproduction. Annu. Rev. Ecol. Evol. Syst. **40**: 127–149.

Engelstädter, J. and G. D. D. Hurst, 2006a Can maternally transmitted endosymbionts facilitate the evolution of haplodiploidy? J. Evol. Biol. **19**: 194–202.

Engelstädter, J. and G. D. D. Hurst, 2006b The dynamics of parasite incidence across host species. Evol. Ecol. **20**: 603–616.

Engelstädter, J., A. Telschow, and P. Hammerstein, 2004 Infection dynamics of different *Wolbachia*-types within one host population. J. Theor. Biol. **231**: 345–355.

Erkosar, B., G. Storelli, A. Defaye, and F. Leulier, 2013 Host-intestinal microbiota mutualism: "learning on the fly". Cell Host Microbe **13**: 8–14.

Esterházy, D., M. S. King, G. Yakovlev, and J. Hirst, 2008 Production of reactive oxygen species by complex I (NADH: ubiquinone

oxidoreductase) from *Escherichia coli* and comparison to the enzyme from mitochondria. Biochemistry **47**: 3964–3971.

Estrela, S., B. Kerr, and J. J. Morris, 2016 Transitions in individuality through symbiosis. Curr. Opin. Microbiol. **31**: 191–198.

Evans, J. D., K. Aronstein, Y. P. Chen, C. Hetru, J.-L. Imler, H. Jiang, M. Kanost, *et al.*, 2006 Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Mol. Biol. **15**: 645–656.

Evison, S. E. F., K. E. Roberts, L. Laurenson, S. Pietravalle, J. Hui, J. C. Biesmeijer, J. E. Smith, G. Budge, and W. O. H. Hughes, 2012 Pervasiveness of parasites in pollinators. PLoS ONE **7**: e30641.

Ewald, P. W., 1987 Transmission modes and evolution of the parasitism-mutualism continuum. Ann. N. Y. Acad. Sci. **503**: 295–306.

Faherty, C. S. and A. T. Maurelli, 2008 Staying alive: bacterial inhibition of apoptosis during infection. Trends Microbiol **16**: 173–180.

Faria, V. G., T. F. Paulo, and É. Sucena, 2016 Testing cannibalism as a mechanism for horizontal transmission of *Wolbachia* in *Drosophila*. Symbiosis **68**: 79–85.

Faria, V. G. and É. Sucena, 2013 *Wolbachia* in the Malpighian tubules: evolutionary dead-end or adaptation? J. Exp. Zool. (Mol. Dev. Evol.) **320B**: 195–199.

Fast, E. M., M. E. Toomey, K. Panaram, D. Desjardins, E. D. Kolaczyk, and H. M. Frydman, 2011 *Wolbachia* enhance *Drosophila* stem cell proliferation and target the germline stem cell niche. Science **334**: 990–992.

Fenton, A., K. N. Johnson, J. C. Brownlie, and G. D. D. Hurst, 2011 Solving the *Wolbachia* paradox: Modeling the tripartite interaction between host, *Wolbachia*, and a natural enemy. Am. Nat. **178**: 333–342.

Ferguson, H. M. and A. F. Read, 2002 Why is the effect of malaria parasites on mosquito survival still unresolved? Trends Parasitol. **18**: 256–261.

Ferree, P. M., H. M. Frydman, J. M. Li, J. Cao, E. Wieschaus, and W. Sullivan, 2005 *Wolbachia* utilizes host microtobules and dynein for anterior localization in the *Drosophila* oocyte. PLoS Pathog. **1**: e14.

Ferreira, Á. G., H. Naylor, S. S. Esteves, I. S. Pais, N. E. Martins, and L. Teixeira, 2014 The Toll-Dorsal pathway is required for resistance to viral oral infection in *Drosophila*. PLoS Pathog. **10**: e1004507.

Fine, P. E. M., 1975 Vectors and vertical transmission: an epidemiologic perspective. Ann. N. Y. Acad. Sci. **266**: 173–194.

Fine, P. E. M., 1978 On the dynamics of symbiote-dependent cytoplasmic incompatibility in culicine mosquitoes. J. Invertebr. Pathol. **30**: 10–18.

Finkel, T., 2011 Signal transduction by reactive oxygen species. J. Cell Biol. **194**: 7–15.

Fisher, R. A., 1930 *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.

Flora, G., D. Gupta, and A. Tiwari, 2012 Toxicity of lead: a review with recent updates. Interdiscip. Toxicol. **5**: 47–58.

Flores, H. A., J. E. Bubnell, C. F. Aquadro, and D. A. Barbash, 2015 The *Drosophila bag of marbles* gene interacts genetically with *Wolbachia* and shows female-specific effects of divergence. PLoS Genet. **11**: e1005453.

Forbes, V. E., 2000 Is hormesis an evolutionary expectation? Funct. Ecol. **14**: 12–24.

Ford, S. A. and K. C. King, 2016 Harnessing the power of defensive microbes: evolutionary implications in nature and disease control. PLoS Pathog. **12**: e1005465.

Foster, J., M. Ganatra, I. Kamal, J. Ware, K. Makarova, N. Ivanova, A. Bhattacharyya, *et al.*, 2005 The *Wolbachia* genome of *Brugia malayi*: Endosymbiont evolution within a human pathogenic nematode. PLoS Biol. **3**: e121.

Frago, E., M. Dicke, and H. C. J. Godfray, 2012 Insect symbionts as hidden players in insect-plant interactions. Trends Ecol. Evol. **27**: 705–711.

Frank, S. A., 1997 Cytoplasmic incompatibility and population structure. J. Theor. Biol. **184**: 327–330.

Freeland, S. J. and B. K. McCabe, 1997 Fitness compensation and the evolution of selfish cytoplasmic elements. Heredity **78**: 391–402.

Frentiu, F. D., J. Robinson, P. R. Young, E. A. McGraw, and S. L. O'Neill, 2010 *Wolbachia*-mediated resistance to dengue virus infection and death at the cellular level. PLoS ONE **5**: e13398.

Frost, C. L., H. Fernández-Marín, J. E. Smith, and W. O. H. Hughes, 2010 Multiple gains and losses of *Wolbachia* symbionts across a tribe of fungus-growing ants. Mol. Ecol. **19**: 4077–4085.

Frost, C. L., S. W. Pollock, J. E. Smith, and W. O. H. Hughes, 2014 *Wolbachia* in the flesh: symbiont intensities in germ-line and somatic tissues challenge the conventional view of *Wolbachia* transmission routes. PLoS ONE **9**: e95122.

Fry, A. J., M. R. Palmer, and D. M. Rand, 2004 Variable fitness effects of *Wolbachia* infection in *Drosophila melanogaster*. Heredity **93**: 379–389.

Fry, A. J. and D. M. Rand, 2002 *Wolbachia* interactions that determine *Drosophila melanogaster* survival. Evolution **56**: 1976–1981.

Frydman, H. M., J. M. Li, D. N. Robson, and E. Wieschaus, 2006 Somatic stem cell niche tropism in *Wolbachia*. Nature **441**: 509–512.

Fukui, T., M. Kawamoto, K. Shoji, T. Kiuchi, S. Sugano, T. Shimada, Y. Suzuki, and S. Katsuma, 2015 The endosymbiotic bacterium *Wolbachia* selectively kills male hosts by targeting the Masculinizing gene. PLoS Pathog. **11**: e1005048.

Fytrou, A., P. G. Schofield, A. R. Kraaijeveld, and S. F. Hubbard, 2006 *Wolbachia* infection suppresses both host defence and parasitoid counter-defence. Proc. R. Soc. B **273**: 791–796.

Gavotte, L., D. R. Mercer, J. J. Stoeckle, and S. L. Dobson, 2010 Costs and benefits of *Wolbachia* infection in immature *Aedes albopictus* depend upon sex and competition level. J. Invertebr. Pathol. **105**: 341–346.

Gems, D. and L. Partridge, 2008 Stress-response hormesis and aging: "that which does not kill us makes us stronger". Cell Metab. **7**: 200–203.

Gerardo, N. M., B. Altincicek, C. Anselme, H. Atamian, S. M. Barribeau, M. de Vos, E. J. Duncan, *et al.*, 2010 Immunity and other defenses in pea aphids, *Acyrthosiphon pisum*. Genome Biol. **11**: R21.

Gerardo, N. M. and B. J. Parker, 2014 Mechanisms of symbiont-conferred protection against natural enemies: an ecological and evolutionary framework. Curr. Opin. Insect Sci. **4**: 8–14.

Gerschman, R., D. L. Gilbert, S. W. Nye, P. Dwyer, and W. O. Fenn, 1954 Oxygen poisoning and X-irradiation: a mechanism in common. Science **119**: 623–626.

Gerth, M., 2016 Classification of *Wolbachia* (Alphaproteobacteria, Rickettsiales): no evidence for a distinct supergroup in cave spiders. Infect. Genet. Evol. **43**: 378–380.

Gerth, M. and C. Bleidorn, 2016 Comparative genomics provides a timeframe for *Wolbachia* evolution and exposes a recent biotin synthesis operon transfer. Nat. Microbiol. **2**: 16241.

Gerth, M., M.-T. Gansauge, A. Weigert, and C. Bleidorn, 2014 Phylogenomic analyses uncover origin and spread of the *Wolbachia* pandemic. Nat. Commun. **5**: 5117.

Gerth, M., J. Röthe, and C. Bleidorn, 2013 Tracing horizontal *Wolbachia* movements among bees (Anthophila): a combined approach using multilocus sequence typing data and host phylogeny. Mol. Ecol. **22**: 6149–6162.

Ghedin, E., S. Wang, D. Spiro, E. Caler, Q. Zhao, J. Crabtree, J. E. Allen, *et al.*, 2007 Draft genome of the filarial nematode parasite *Brugia malayi*. Science **317**: 1756–1760.

Gilbert, S. F., E. McDonald, N. Boyle, N. Buttino, L. Gyi, M. Mai, N. Prakash, and J. Robinson, 2010 Symbiosis as a source of selectable epigenetic variation: taking the heat for the big guy. Phil. Trans. R. Soc. B **365**: 671–678.

Gill, A. C., A. C. Darby, and B. L. Makepeace, 2014 Iron necessity: the secret of *Wolbachia*'s success? PLoS Negl. Trop. Dis. **8**: e3224.

Gillespie, D. T., 1977 Exact stochastic simulation of coupled chemical reactions. J. Phys. Chem. **81**: 2340–2361.

Giordano, R., S. L. O'Neill, and H. M. Robertson, 1995 *Wolbachia* infections and the expression of cytoplasmic incompatibility in *Drosophila sechellia* and *D. mauritiana*. Genetics **140**: 1307–1317.

Girin, C. and M. Boulétreau, 1995 Microorganism-associated variation in host infestation efficiency in a parasitoid wasp, *Trichogramma bourarachae* (Hymenoptera: Trichogrammatidae). Experientia **51**: 398–401.

Glaser, R. L. and M. A. Meola, 2010 The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. PLoS ONE **5**: e11977.

Glowska, E., A. Dragun-Damian, M. Dabert, and M. Gerth, 2015 New *Wolbachia* supergroups detected in quill mites (Acari: Syringophilidae). Infect. Genet. Evol. **30**: 140–146.

Gomariz-Zilber, E. and M. Thomas-Orillard, 1993 *Drosophila* C virus and *Drosophila* hosts: a good association in various environments. J. Evol. Biol. **6**: 677–689.

Gomes, L. C. and I. Dikic, 2014 Autophagy in antimicrobial immunity. Mol. Cell **54**: 224–233.

Goto, S., H. Anbutsu, and T. Fukatsu, 2006 Asymmetrical interactions between *Wolbachia* and *Spiroplasma* endosymbionts coexisting in the same insect host. Appl. Environ. Microbiol. **72**: 4805–4810.

Gottlieb, Y. and E. Zchori-Fein, 2001 Irreversible thelytokous reproduction in *Muscidifurax uniraptor*. Entomol. Exp. Appl. **100**: 271–278.

Gottlieb, Y., E. Zchori-Fein, J. H. Werren, and T. L. Karr, 2002 Diploidy restoration in *Wolbachia*-infected *Muscidifurax uniraptor* (Hymenoptera: Pteromalidae). J. Invertebr. Pathol. **81**: 166–174.

Graça-Souza, A. V., C. Maya-Monteiro, G. O. Paiva-Silva, G. R. C. Braz, M. C. Paes, M. H. F. Sorgine, *et al.*, 200 Adaptations against heme toxicity in blood-feeding arthropods. Insect Biochem. Mol. Biol. **36**: 322–335.

Graham, R. I., D. Grzywacz, W. L. Mushobozi, and K. Wilson, 2012 *Wolbachia* in a major African crop pest increases susceptibility to viral disease rather than protects. Ecol. Lett. **15**: 993–1000.

Grenier, S., S. M. Gomes, B. Pintureau, F. Lassablière, and P. Bolland, 2002 Use of tetracycline in larval diet to study the effect of *Wolbachia* on host fecundity and clarify taxonomic status of *Trichogramma* species in cured bisexual lines. J. Invertebr. Pathol. **80**: 13–21.

Ha, E.-M., K.-A. Lee, S. H. Park, S.-H. Kim, H.-J. Nam, H.-Y. Lee, *et al.*, 2009a Regulation of DUOX by the Gαq-phospholipase Cβ-Ca2+ pathway in *Drosophila* gut immunity. Dev. Cell **16**: 386–397.

Ha, E.-M., K.-A. Lee, Y. Y. Seo, S.-H. Kim, J.-H. Lim, B.-H. Oh, *et al.*, 2009b Coordination of multiple dual oxidase-regulatory pathways in responses to commensal and infectious microbes in *Drosophila* gut. Nat. Immunol. **10**: 949–957.

Ha, E.-M., C.-T. Oh, Y. S. Bae, and W.-J. Lee, 2005a A direct role for dual oxidase in *Drosophila* gut immunity. Science **310**: 847–850.

Ha, E.-M., C.-T. Oh, J.-H. Ryu, Y.-S. Bae, S.-W. Kang, I.-H. Jang, *et al.*, 2005b An antioxidant system required for host protection against gut infection in *Drosophila*. Dev. Cell **8**: 125–132.

Habayeb, M. S., R. Cantera, G. Casanova, J.-O. Ekström, S. Albright, and D. Hultmark, 2009 The *Drosophila* Nora virus is an enteric virus, transmitted via feces. J. Invertebr. Pathol. **101**: 29–33.

Hadfield, S. J. and J. M. Axton, 1999 Germ cells colonized by endosymbiotic bacteria. Nature **402**: 482.

Hafer, N. and N. Pike, 2010 Shape change in viable eggs of the collembolan *Folsomia candida* provides insight into the role of *Wolbachia* endosymbionts. Zool. Res. **31**: 623–626.

Haine, E. R., 2008 Symbiont-mediated protection. Proc. R. Soc. B **275**: 353–361.

Haine, E. R. and J. M. Cook, 2005 Convergent incidences of *Wolbachia* infection in fig wasp communities from two continents. Proc. R. Soc. Lond. B **272**: 421–429.

Haine, E. R., N. J. Pickup, and J. M. Cook, 2005 Horizontal transmission of *Wolbachia* in a *Drosophila* community. Ecol. Entomol. **30**: 464–472.

Hamanaka, R. B. and N. S. Chandel, 2010 Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. Trends Biochem. Sci. **35**: 505–513.

Hamm, C. A., D. J. Begun, A. Vo, C. C. R. Smith, P. Saelao, A. O. Shaver, J. Jaenike, and M. Turelli, 2014 *Wolbachia* do not live by

reproductive manipulation alone: infection polymorphism in *Drosophila suzukii* and *D. subpulchrella*. Mol. Ecol. **23**: 4871–4885.

Hariri, A. R., J. H. Werren, and G. S. Wilkinson, 1998 Distribution and reproductive effects of *Wolbachia* in stalk-eyed flies (Diptera: Diopsidae). Heredity **81**: 254–260.

Harman, D., 1956 Aging: a theory based on free radical and radiation chemistry. J. Gerontol. **11**: 298–300.

Harman, D., 1972 The biologic clock: the mitochondria? J. Am. Geriatr. Soc. **20**: 145–147.

Haukedal, J. A., 2013 *Regulation of Wolbachia Density Within Drosophila Simulans*. Ph.D. thesis, University of Alberta, Edmonton, Alberta.

Haygood, R. and M. Turelli, 2009 Evolution of incompatibility-inducing microbes in subdivided host populations. Evolution **63**: 432–447.

Heath, B. D., R. D. Butcher, W. G. Whitfield, and S. F. Hubbard, 1999 Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a naturally occurring mechanism. Curr. Biol. **9**: 313–316.

Hedges, L. M., J. C. Brownlie, S. L. O'Neill, and K. N. Johnson, 2008 *Wolbachia* and virus protection in insects. Science **322**: 702.

Hedges, L. M. and K. N. Johnson, 2008 Induction of host defence responses by *Drosophila* C virus. J. Gen. Virol. **89**: 1497–1501.

Hedges, L. M., R. Yamada, S. L. O'Neill, and K. N. Johnson, 2012 The small interfering RNA pathway is not essential for *Wolbachia*-mediated antiviral protection in *Drosophila melanogaster*. Appl. Environ. Microbiol. **78**: 6773–6776.

Herre, E. A., N. Knowlton, U. G. Mueller, and S. A. Rehner, 1999 The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends Ecol. Evol. **14**: 49–53.

Hertig, M., 1936 The rickettsia, *Wolbachia pipientis* (gen. et sp.n.) and associated inclusions of the mosquito, *Culex pipiens*. Parasitology **28**: 453–486.

Hertig, M. and S. B. Wolbach, 1924 Studies on rickettsia-like microorganisms in insects. J. Med. Res. **44**: 329–374.

Hetru, C. and J. A. Hoffmann, 2009 NF-κB in the immune response of *Drosophila*. Cold Spring Harb. Perspect. Biol. **1**: a000232.

Higham, D. J. and N. J. Higham, 2005 *MATLAB Guide*. Society for Industrial and Applied Mathematics, Philadelphia, PA, second edition.

Hilgenböcker, K., P. Hammerstein, P. Schlattmann, A. Telschow, and J. H. Werren, 2008 How many species are infected with *Wolbachia*?–A statistical analysis of current data. FEMS Microbiol. Lett. **281**: 215–220.

Hiroki, M., Y. Kato, T. Kamito, and K. Miura, 2002 Feminization of genetic males by a symbiotic bacterium in a butterfly, *Eurema hecabe* (Lepidoptera: Pieridae). Naturwissenschaften **89**: 167–170.

Hirose, M., P. Schilf, Y. Gupta, M. N. Wright, O. Jöhren, A. E. Wagner, *et al.*, 2016 Lifespan effects of mitochondrial mutations. Nature **540**: E13–E14.

Hoffmann, A. A., D. Clancy, and J. Duncan, 1996 Naturally-occurring *Wolbachia* infection in *Drosophila simulans* that does not cause cytoplasmic incompatibility. Heredity **76**: 1–8.

Hoffmann, A. A., M. Hercus, and H. Dagher, 1998 Population dynamics of the *Wolbachia* infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. Genetics **148**: 221–231.

Hoffmann, A. A. and M. Turelli, 1997 Cytoplasmic incompatibility in insects. In *Influential Passengers. Inherited Microorganisms and Arthropod Reproduction*, edited by S. L. O'Neill, A. A. Hoffmann, and J. H. Werren, pp. 42–80, Oxford University Press, Oxford, UK.

Hoffmann, A. A., M. Turelli, and L. G. Harshman, 1990 Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. Genetics **126**: 933–948.

Hornett, E. A., S. Charlat, A. M. Duplouy, N. Davies, G. K. Roderick, N. Wedell, and G. D. Hurst, 2006 Evolution of male-killer suppression in a natural population. PLoS Biol. **4**: e283.

Hornett, E. A., A. M. Duplouy, N. Davies, G. K. Roderick, N. Wedell, G. D. Hurst, and S. Charlat, 2008 You can't keep a good parasite down: evolution of a male-killer suppressor uncovers cytoplasmic incompatibility. Evolution **62**: 1258–1263.

Hosokawa, T., R. Koga, Y. Kikuchi, X.-Y. Meng, and T. Fukatsu, 2010 *Wolbachia* as a bacteriocyte-associated nutritional mutualist. Proc. Natl. Acad. Sci. U.S.A. **107**: 769–774.

Huang, J., V. Canadien, G. Y. Lam, B. E. Steinberg, M. C. Dinauer, M. A. O. Magalhaes, *et al.*, 2009 Activation of antibacterial autophagy by NADPH oxidases. Proc. Natl. Acad. Sci. U.S.A. **106**: 6226–6231.

Hughes, G. L., P. G. Allsopp, S. M. Brumbley, M. Woolfit, E. A. McGraw, and S. L. O'Neill, 2011a Variable infection frequency and high diversity of multiple strains of *Wolbachia pipientis* in *Perkinsiella* planthoppers. Appl. Environ. Microbiol. **77**: 2165–2168.

Hughes, G. L., B. L. Dodson, R. M. Johnson, C. C. Murdock, H. Tsujimoto, Y. Suzuki, A. A. Patt, *et al.*, 2014 Native microbiome impedes vertical transmission of *Wolbachia* in *Anopheles* mosquitoes. Proc. Natl. Acad. Sci. U.S.A. **111**: 12498–12503.

Hughes, G. L., R. Koga, P. Xue, T. Fukatsu, and J. L. Rasgon, 2011b *Wolbachia* infections are virulent and inhibit the human Malaria parasite *Plasmodium falciparum* in *Anopheles gambiae*. PLoS Pathog. **7**: e1002043.

Hughes, G. L., X. Ren, J. L. Ramirez, J. M. Sakamoto, J. A. Bailey, A. E. Jedlicka, *et al.*, 2011c *Wolbachia* infections in *Anopheles gambiae* cells: transcriptomic characterization of a novel host-smbiont interaction. PLoS Pathog. **7**: e1001296.

Hughes, G. L., J. Vega-Rodriguez, P. Xue, and J. L. Rasgon, 2012 *Wolbachia* strain *w*AlbB enhances infection by the rodent malaria parasite *Plasmodium berghei* in *Anopheles gambiae* mosquitoes. Appl. Environ. Microbiol. **78**: 1491–1495.

Huigens, M. E., 2003 *On the Evolution of Wolbachia-induced Parthenogenesis in Trichogramma Wasps*. Ph.D. thesis, Wageningen University, Wageningen, Netherlands.

Huigens, M. E., R. P. de Almeida, P. A. Boons, R. F. Luck, and R. Stouthamer, 2004 Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. Proc. R. Soc. Lond. B **271**: 509–515.

Huigens, M. E. and R. Stouthamer, 2003 Parthenogenesis associated with *Wolbachia*. In *Insect Symbiosis*, edited by K. Bourtzis and T. A. Miller, pp. 247–266, CRC Press, Boca Raton.

Hurst, G. D. and C. L. Frost, 2015 Reproductive parasitism: maternally inherited symbionts in a biparental world. Cold Spring Harb. Perspect. Biol. **7**: a017699.

Hurst, G. D., F. M. Jiggins, and A. Pomiankowski, 2002 Which way to manipulate host reproduction? *Wolbachia* that cause cytoplasmic incompatibility are easily invaded by sex ratio-distorting mutants. Am. Nat. **160**: 360–373.

Hurst, G. D. D., L. D. Hurst, and M. E. N. Majerus, 1992 Selfish genes move sideways. Nature **356**: 659–660.

Hurst, G. D. D. and F. M. Jiggins, 2005 Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc. R. Soc. B **272**: 1525–1534.

Hurst, G. D. D., F. M. Jiggins, and M. E. N. Majerus, 2003 Inherited microorganisms that selectively kill male hosts: the hidden players of insect evolution? In *Insect Symbiosis*, edited by K. Bourtzis and T. A. Miller, pp. 177–198, CRC Press, Boca Raton.

Hurst, G. D. D., A. P. Johnson, J. H. G. von der Schulenburg, and Y. Fuyama, 2000 Male-killing *Wolbachia* in *Drosophila*: a temperature-sensitive trait with a threshold bacterial density. Genetics **156**: 699–709.

Hurst, L. D., 1991a The evolution of cytoplasmic incompatibility or when spite can be successful. J. Theor. Biol. **148**: 269–277.

Hurst, L. D., 1991b The incidences and evolution of cytoplasmic male killers. Proc. R. Soc. Lond. B **244**: 91–99.

Hurst, L. D., 1992 Intragenomic conflict as an evolutionary force. Proc. R. Soc. Lond. B **248**: 135–140.

Hurst, L. D., A. Atlan, and B. O. Bengtsson, 1996 Genetic conflicts. Q. Rev. Biol. **71**: 317–364.

Hurst, L. D. and G. T. McVean, 1996 Clade selection, reversible evolution and the persistence of selfish elements: the evolutionary dynamics of cytoplasmic incompatibility. Proc. R. Soc. Lond. B **263**: 97–104.

Hutchence, K. J., B. Fischer, S. Paterson, and G. D. D. Hurst, 2011 How do insects react to novel inherited symbionts? A microarray analysis of *Drosophila melanogaster* response to the presence of natural and introduced *Spiroplasma*. Mol. Ecol. **20**: 950–958.

Ijichi, N., N. Kondo, R. Matsumoto, M. Shimada, H. Ishikawa, and T. Fukatsu, 2002 Internal spatiotemporal population dynamics of infection with three *Wolbachia* strains in the adzuki bean beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Appl. Environ. Microbiol. **68**: 4074–4080.

Isaksson, C., B. C. Sheldon, and T. Uller, 2011 The challenges of integrating oxidative stress into life-history biology. Bioscience **61**: 194–202.

Iturbe-Ormaetxe, I., T. Walker, and S. L. O'Neill, 2011 *Wolbachia* and the biological control of mosquito-borne disease. EMBO Rep. **12**: 508–518.

Jacquet, A., B. Horard, and B. Loppin, 2017 Does pupal communication influence *Wolbachia*-mediated cytoplasmic incompatibility? Curr. Biol. **27**: R53–R55.

Jaenike, J., 2007 Spontaneous emergence of a new *Wolbachia* phenotype. Evolution **61**: 2244–2252.

Jaenike, J., 2012 Population genetics of beneficial heritable symbionts. Trends Ecol. Evol. **27**: 226–232.

Jaenike, J. and K. A. Dyer, 2008 No resistance to male-killing *Wolbachia* after thousands of years of infection. J. Evol. Biol. **21**: 1570–1577.

Jaenike, J., M. Polak, A. Fiskin, M. Helou, and M. Minhas, 2007 Interspecific transmission of endosymbiotic *Spiroplasma* by mites. Biol. Lett. **3**: 23–25.

Jaenike, J., R. Unckless, S. N. Cockburn, L. M. Boelio, and S. J. Perlman, 2010 Adaptation via symbiosis: recent spread of a *Drosophila* defensive symbiont. Science **329**: 212–215.

Jansen, V. A. A., M. Turelli, and H. C. F. Godfray, 2008 Stochastic spread of *Wolbachia*. Proc. R. Soc. B **275**: 2769–2776.

Jeney, V., J. Balla, A. Yachie, Z. Varga, G. M. Vercellotti, J. W. Eaton, *et al.*, 2002 Pro-oxidant and cytotoxic effects of circulating heme. Blood **100**: 879–887.

Jeong, G. and R. Stouthamer, 2005 Genetics of female functional virginity in the Parthenogenesis-*Wolbachia* infected parasitoid wasp *Telenomus nawai* (Hymenoptera: Scelionidae). Heredity **94**: 402–407.

Jiggins, F. M., 2003 Male-killing *Wolbachia* and mitochondrial DNA: selective sweeps, hybrid introgression and parasite population dynamics. Genetics **164**: 5–12.

Jiggins, F. M., 2016 Open questions: how does *Wolbachia* do what it does? BMC Biol. **14**: 92.

Jiggins, F. M., J. K. Bentley, M. E. Majerus, and G. D. Hurst, 2002a Recent changes in phenotype and patterns of host specialization in *Wolbachia* bacteria. Mol. Ecol. **11**: 1275–1283.

Jiggins, F. M. and G. D. D. Hurst, 2011 Rapid insect evolution by symbiont transfer. Science **332**: 185–186.

Jiggins, F. M., G. D. D. Hurst, and Z. Yang, 2002b Hosts-symbiont conflicts: positive selection on an outer membrane protein of parasitic but not mutualistic Rickettsiaceae. Mol. Biol. Evol. **19**: 1341–1349.

Jiggins, F. M., J. P. Randerson, G. D. D. Hurst, and M. E. N. Majerus, 2002c How can sex ratio distorters reach extreme prevalences? Male-killing *Wolbachia* are not suppressed and have near-perfect vertical transmission efficiency in *Acraea encedon*. Evolution **56**: 2290–2295.

Johnson, K. N., 2015 Bacteria and antiviral immunity in insects. Curr. Opin. Insect Sci. **8**: 97–103.

Jones, E. O., A. White, and M. Boots, 2007 Interference and the persistence of vertically transmitted parasites. J. Theor. Biol. **246**: 10–17.

Jones, E. O., A. White, and M. Boots, 2011 The evolution of host protection by vertically transmitted parasites. Proc. R. Soc. B **278**: 863–870.

Kageyama, D., S. Narita, and M. Watanabe, 2012 Insect sex determination manipulated by their endosymbionts: incidences, mechanisms and implications. Insects **3**: 161–199.

Kageyama, D., M. Ohno, T. Sasaki, A. Yoshido, T. Konagaya, A. Jouraku, S. Kuwazaki, *et al.*, 2017 Master manipulation continued: feminizing *Wolbachia* endosymbiont distorts sex chromosome inheritance. bioRxiv p. doi:10.1101/115386.

Kageyama, D. and W. Traut, 2004 Opposite sex-specific effects of *Wolbachia* and interference with the sex determination of its host *Ostrinia scapulalis*. Proc. R. Soc. Lond. B **271**: 251–258.

Kaiser, W., E. Huguet, J. Casas, C. Commin, and D. Giron, 2010 Plant green-island phenotype induced by leaf-miners is mediated by bacterial symbionts. Proc. R. Soc. B **277**: 2311–2319.

Kambris, Z., A. M. Blagborough, S. B. Pinto, M. S. C. Blagrove, H. C. J. Godfray, R. E. Sinden, and S. P. Sinkins, 2010 *Wolbachia* stimulates immune gene expression and inhibits *Plasmodium* development in *Anopheles gambiae*. PLoS Pathogens **6**: e1001143.

Kambris, Z., P. E. Cook, H. K. Phuc, and S. P. Sinkins, 2009 Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. Science **326**: 134–136.

Kaneko, T., T. Yano, K. Aggarwal, J.-H. Lim, K. Ueda, Y. Oshima, *et al.*, 2006 PGRP-LC and PGRP-LE have essential yet distinct functions in the *Drosophila* immune response to monomeric DAP-type peptidoglycan. Nat. Immunol. **7**: 715–723.

Keeling, M. J. and P. Rohani, 2008 *Modeling Infectious Diseases in Humans and Animals*. Princeton University Press, Princeton, NJ.

Keller, G. P., D. M. Windsor, J. M. Saucedo, and J. H. Werren, 2004 Reproductive effects and geographical distributions of two *Wolbachia* strains infecting the Neotropical beetle, *Chelymorpha alternans* Boh. (Chrysomelidae, Cassidinae). Mol. Ecol. **13**: 2405–2420.

Kern, P., J. M. Cook, D. Kageyama, and M. Riegler, 2015 Double trouble: combined action of meiotic drive and *Wolbachia* feminization in *Eurema* butterflies. Biol. Lett. **11**: 20150095.

Kharade, S. V., N. Mittal, S. P. Das, P. Sinha, and N. Roy, 2005 Mrg19 depletion increases *S. cerevisiae* lifespan by augmenting ROS defence. FEBS Lett. **579**: 6809–6813.

Kiers, E. T. and S. A. West, 2015 Evolving new organisms via symbiosis. Science **348**: 392–394.

King, K. C. and G. D. D. Hurst, 2010 Losing the desire: selection can promote obligate asexuality. BMC Biol. **8**: 101.

Kirkwood, T. B. L. and A. Kowald, 2012 The free-radical theory of ageing–older, wiser and still alive. Bioessays **34**: 692–700.

Kittayapong, P., W. Jamnongluk, A. Thipaksorn, J. R. Milne, and C. Sindhusake, 2003 *Wolbachia* infection complexity among insects in the tropical rice-field community. Mol. Ecol. **12**: 1049–1060.

Kiuchi, T., H. Koga, M. Kawamoto, K. Shoji, H. Sakai, Y. Arai, G. Ishihara, *et al.*, 2014 A single female-specific piRNA is the primary determiner of sex in the silkworm. Nature **509**: 633–636.

Koehncke, A., A. Telschow, J. H. Werren, and P. Hammerstein, 2009 Life and death of an influential passenger: *Wolbachia* and the evolution of CI-modifiers by their hosts. PLoS ONE **4**: e4425.

Kondo, N., M. Tuda, Y. Toquenaga, Y.-C. Lan, S. Buranapanichpan, S.-B. Horng, M. Shimada, and T. Fukatsu, 2011 *Wolbachia* infections in world populations of bean beetles (Coleoptera: Chrysomelidae: Bruchinae) infesting cultivated and wild legumes. Zool. Sci. **28**: 501–508.

Kose, H. and T. L. Karr, 1995 Organization of *Wolbachia pipientis* in the *Drosophila* fertilized egg and embryo revealed by an anti-*Wolbachia* monoclonal antibody. Mech. Dev. **51**: 275–288.

Koukou, K., H. Pavlikaki, G. Kilias, J. H. Werren, K. Bourtzis, and S. N. Alahiotis, 2006 Influence of antibiotic treatment and *Wolbachia* curing on sexual isolation among *Drosophila melanogaster* cage populations. Evolution **60**: 87–96.

Kremer, N., D. Charif, H. Henri, M. Bataille, G. Prévost, K. Kraaijeveld, and F. Vavre, 2009a A new case of *Wolbachia* dependence in the genus *Asobara*: evidence for parthenogenesis induction in *Asobara japonica*. Heredity **103**: 248–256.

Kremer, N., D. Charif, H. Henri, F. Gavory, P. Wincker, P. Mavingui, and F. Vavre, 2012 Influence of *Wolbachia* on host

gene expression in an obligatory symbiosis. BMC Microbiol. **12** (Suppl. 1): S7.

Kremer, N., F. Dedeine, D. Charif, C. Finet, R. Allemand, and F. Vavre, 2010 Do variable compensatory mechanisms explain the polymorphism of the dependence phenotype in the *Asobara tabida-Wolbachia* association? Evolution **64**: 2969–2979.

Kremer, N., D. Voronin, D. Charif, P. Mavingui, B. Mollereau, and F. Vavre, 2009b *Wolbachia* interferes with ferritin expression and iron metabolism in insects. PLoS Pathogens **5**: e1000630.

Kriesner, P., W. R. Conner, A. R. Weeks, M. Turelli, and A. A. Hoffmann, 2016 Persistence of a *Wolbachia* infection frequency cline in *Drosophila melanogaster* and the possible role of reproductive dormancy. Evolution **70**: 979–997.

Kriesner, P., A. A. Hoffmann, S. F. Lee, M. Turelli, and A. R. Weeks, 2013 Rapid sequential spread of two *Wolbachia* variants in *Drosophila simulans*. PLoS Pathog. **9**: e1003607.

Kuijper, B. and I. Pen, 2010 The evolution of haplodiploidy by male-killing endosymbionts: importance of population structure and endosymbiont mutualisms. J. Evol. Biol. **23**: 40–52.

Kumar, S., G. K. Christophides, R. Cantera, B. Charles, Y. S. Han, S. Meister, *et al.*, 2003 The role of reactive oxygen species on *Plasmodium* melanotic encapsulation in *Anopheles gambiae*. Proc. Natl. Acad. Sci. U.S.A. **100**: 14139–14144.

Kuraishi, T., A. Hori, and S. Kurata, 2013 Host-microbe interactions in the gut of *Drosophila melanogaster*. Front. Physiol. **4**: 375.

Kurz, M., I. Iturbe-Ormaetxe, R. Jarrott, S. R. Shouldice, M. A. Wouters, P. Frei, *et al.*, 2009 Structural and functional characterization of the oxidoreductase α-DsbA1 from *Wolbachia pipientis*. Antioxid. Redox Signal. **11**: 1485–1500.

Lambrechts, L. and T. W. Scott, 2009 Mode of transmission and the evolution of arbovirus virulence in mosquito vectors. Proc. R. Soc. B **276**: 1369–1378.

Lamiable, O. and J.-L. Imler, 2014 Induced antiviral innate immunity in *Drosophila*. Curr. Opin. Microbiol. **20**: 62–68.

Landmann, F., G. A. Orsi, B. Loppin, and W. J. Sullivan, 2009 *Wolbachia*-mediated cytoplasmic incompatibility is associated with impaired histone deposition in the male pronucleus. PLoS Pathog. **5**: e1000343.

Landmann, F., D. Voronin, W. Sullivan, and M. J. Taylor, 2011 Anti-filarial activity of antibiotic therapy is due to extensive apoptosis after *Wolbachia* depletion from filarial nematodes. PLoS Pathog. **7**: e1002351.

Lapointe, J. and S. Hekimi, 2010 When a theory of aging ages badly. Cell. Mol. Life Sci. **67**: 1–8.

Laven, H., 1951 Crossing experiments with *Culex* strains. Evolution **5**: 370–375.

Laven, H., 1956 Cytoplasmic inheritance in *Culex*. Nature **177**: 141–142.

Law, R. and U. Dieckmann, 1998 Symbiosis through exploitation and the merger of lineages in evolution. Proc. R. Soc. Lond. B **265**: 1245–1253.

Le Clec'h, W., C. Braquart-Varnier, M. Raimond, J.-B. Ferdy, D. Bouchon, and M. Sicard, 2012 High virulence of *Wolbachia* after host switching: when autophagy hurts. PLoS Pathog. **8**: e1002844.

Le Clec'h, W., F. D. Chevalier, L. Genty, J. Bertaux, D. Bouchon, and M. Sicard, 2013 Cannibalism and predation as paths for horizontal passage of *Wolbachia* between terrestrial isopods. PLoS ONE **8**: e60232.

Leclercq, S., J. Thézé, M. A. Chebbi, I. Giraud, B. Moumen, L. Ernenwein, P. Grève, *et al.*, 2016 Birth of a W sex chromosome by horizontal transfer of *Wolbachia* bacterial symbiont genome. Proc. Natl. Acad. Sci. U.S.A. **113**: 15036–15041.

Lee, K.-A., B. Kim, J. Bhin, D. H. Kim, H. You, E.-K. Kim, *et al.*, 2015 Bacterial uracil modulates *Drosophila* DUOX-dependent gut immunity via Hedgehog-induced signaling endosomes. Cell Host Microbe **17**: 191–204.

Lee, K.-A., S.-H. Kim, E.-K. Kim, E.-M. Ha, H. You, B. Kim, *et al.*, 2013 Bacterial-derived uracil as a modulator of mucosal immunity and gut-microbe homeostasis in *Drosophila*. Cell **153**: 797–811.

Lefoulon, E., O. Bain, B. L. Makepeace, C. d'Haese, S. Uni, C. Martin, and L. Gavotte, 2016 Breakdown of coevolution between symbiotic bacteria *Wolbachia* and their filarial hosts. PeerJ **4**: e1840.

Lemaitre, B. and J. Hoffmann, 2007 The host defense of *Drosophila melanogaster*. Annu. Rev. Immunol. **25**: 697–743.

LePage, D. P., J. A. Metcalf, S. R. Bordenstein, J. On, J. I. Perlmutter, J. D. Shropshire, E. M. Layton, *et al.*, 2017 Prophage WO genes recapitulate and enhance *Wolbachia*-induced cytoplasmic incompatibility. Nature **543**: 243–247.

Leulier, F., C. Parquet, S. Pili-Floury, J. H. Ryu, M. Caroff, W.-J. Lee, *et al.*, 2003 The *Drosophila* immune system detects bacteria through specific peptidoglycan recognition. Nat. Immunol. **4**: 478–484.

Li, L. and T. Xie, 2005 Stem cell niche: structure and function. Annu. Rev. Cell Dev. Biol. **21**: 605–631.

Li, S.-J., M. Z. Ahmed, N. Lv, P.-Q. Shi, X.-M. Wang, J.-L. Huang, and B.-L. Qiu, 2016 Plant-mediated horizontal transmission of *Wolbachia* between whiteflies. ISME J. Advance online publication. doi:10.1038/ismej.2016.164 .

Li, Y.-Y., K. D. Floate, P. G. Fields, and B.-P. Pang, 2014 Review of treatment methods to remove *Wolbachia* bacteria from arthropods. Symbiosis **62**: 1–15.

Lipsitch, M., M. A. Nowak, D. Ebert, and R. M. May, 1995 The population dynamics of vertically and horizontally transmitted parasites. Proc. R. Soc. Lond. B **260**: 321–327.

Liu, H., W. Bao, M. Lin, H. Niu, and Y. Rikihisa, 2012 Ehrlichia type IV secretion effector ECH0825 is translocated to mitochondria and curbs ROS and apoptosis by upregulating host MnSOD. Cell. Microbiol. **14**: 1037–1050.

Lively, C. M., K. Clay, M. J. Wade, and C. Fuqua, 2005 Competitive co-existence of vertically and horizontally transmitted parasites. Evol. Ecol. Res. **7**: 1183–1190.

Login, F. H., S. Balmand, A. Vallier, C. Vincent-Monégat, A. Vigneron, M. Weiss-Gayet, *et al.*, 2011 Antimicrobial peptides keep insect endosymbionts under control. Science **334**: 362–365.

Longdon, B., D. K. Fabian, G. D. D. Hurst, and F. M. Jiggins, 2012 Male-killing *Wolbachia* do not protect *Drosophila bifasciata* against viral infection. BMC Microbiol. **12** (Suppl. 1): S8.

Longdon, B., L. Wilfert, J. Osei-Poku, H. Cagney, D. J. Obbard, and F. M. Jiggins, 2011 Host-switching by a vertically transmitted rhabdovirus in *Drosophila*. Biol. Lett. **7**: 747–750.

Loppin, B., R. Dubruille, and B. Horard, 2015 The intimate genetics of *Drosophila* fertilization. Open Biol. **5**: 150076.

Louis, C. and L. Nigro, 1989 Ultrastructural evidence of *Wolbachia* Rickettsiales in *Drosophila simulans* and their relationships with unidirectional cross-incompatibility. J. Invertebr. Pathol. **54**: 39–44.

Lu, P., G. Bian, X. Pan, and Z. Xi, 2012 *Wolbachia* induces density-dependent inhibition to dengue virus in mosquito cells. PLoS Negl. Trop. Dis. **6**: e1754.

Ma, W.-J., B. A. Pannebakker, L. van de Zande, T. Schwander, B. Wertheim, and L. W. Beukeboom, 2015 Diploid males support a two-step mechanism of endosymbiont-induced thelytoky in a parasitoid wasp. BMC Evol. Biol. **15**: 84.

Ma, W.-J., F. Vavre, and L. W. Beukeboom, 2014 Manipulation of arthropod sex determination by endosymbionts: diversity and molecular mechanisms. Sex. Dev. **8**: 59–73.

Majerus, T. M. O. and M. E. N. Majerus, 2010 Intergenomic arms races: detection of a nuclear rescue gene of male-killing in a ladybird. PLoS Pathog. **6**: e1000987.

Mallet, J., 2005 Hybridization as an invasion of the genome. Trends Ecol. Evol. **20**: 229–237.

Martinez, J., R. Cogni, C. Cao, S. Smith, C. J. R. Illingworth, and F. M. Jiggins, 2016 Addicted? Reduced host resistance in populations with defensive symbionts. Proc. R. Soc. B **283**: 20160778.

Martinez, J., B. Longdon, S. Bauer, Y.-S. Chan, W. J. Miller, K. Bourtzis, *et al.*, 2014 Symbionts commonly provide broad spectrum resistance to viruses in insects: a comparative analysis of *Wolbachia* strains. PLoS Pathog **10**: e1004369.

Martinez, J., S. Ok, S. Smith, K. Snoeck, J. P. Day, and F. M. Jiggins, 2015 Should symbionts be nice or selfish? Antiviral effects of *Wolbachia* are costly but reproductive parasitism is not. PLoS Pathog. **11**: e1005021.

Martins, A. B., I. M. Ventura, and L. B. Klaczko, 2010 *Spiroplasma* infection in *Drosophila melanogaster*: What is the advantage of killing males? J. Invertebr. Pathol. **105**: 145–150.

Masri, L. and S. Cremer, 2014 Individual and social immunisation in insects. Trends Immunol. **35**: 471–482.

Mateos, M., S. J. Castrezana, B. J. Nankivell, A. M. Estes, T. A. Markow, and N. A. Moran, 2006 Heritable endosymbionts of *Drosophila*. Genetics **174**: 363–376.

Maynard Smith, J. and E. Szathmáry, 1997 *The Major Transitions in Evolution*. Oxford University Press, Oxford.

McCall, K., 2004 Eggs over easy: cell death in the *Drosophila* ovary. Dev. Biol. **274**: 3–14.

McClure, C. D., W. Zhong, V. L. Hunt, F. M. Chapman, F. V. Hill, and N. K. Priest, 2014 Hormesis results in trade-offs with immunity. Evolution **68**: 2225–2233.

McCord, J. M., B. B. J. Keele, and I. Fridovich, 1971 An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. Proc. Natl. Acad. Sci. U.S.A. **68**: 1024–1027.

McFall-Ngai, M., 2008 Are biologists in 'future shock'? Symbiosis integrates biology across domains. Nat. Rev. Microbiol. **6**: 789–792.

McFall-Ngai, M., M. G. Hadfield, T. C. G. Bosch, H. V. Carey, T. Domazet-Lošo, A. E. Douglas, N. Dubilier, *et al.*, 2013 Animals in a bacterial world, a new imperative for the life sciences. Proc. Natl. Acad. Sci. U.S.A. **110**: 3229–3236.

McKean, K. A. and B. Lazzaro, 2011 The costs of immunity and the evolution of immunological defense mechanisms. In *Mechanisms of Life History Evolution*, edited by T. Flatt and A. Heyland, pp. 299–310, Oxford University Press, Oxford.

McPeek, M. A. and J. M. Brown, 2007 Clade age and not diversification rate explains species richness among animal taxa. Am. Nat. **169**: E97–E106.

Merkling, S. H. and R. P. van Rij, 2013 Beyond RNAi: Antiviral defense strategies in *Drosophila* and mosquito. J. Insect Physiol. **59**: 159–170.

Messens, J., N. Rouhier, and J.-F. Collet, 2013 Redox homeostasis. In *Oxidative Stress and Redox Regulation*, edited by U. Jakob and D. Reichmann, pp. 59–84, Springer, Dordrecht.

Metcalfe, N. B. and C. Alonso-Alvarez, 2010 Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. Funct. Ecol. **24**: 984–996.

Miller, W. J., L. Ehrman, and D. Schneider, 2010 Infectious speciation revisited: impact of symbiont-depletion on female fitness and mating behavior of *Drosophila paulistorum*. PLoS Pathogens **6**: e1001214.

Misof, B., S. Liu, K. Meusemann, R. S. Peters, A. Donath, C. Mayer, P. B. Frandsen, *et al.*, 2014 Phylogenomics resolves the timing and pattern of insect evolution. Nature **346**: 763–767.

Mitsuhashi, W., H. Ikeda, and M. Muraji, 2011 Fifty-year trend towards suppression of *Wolbachia*-induced male-killing by its butterfly host, *Hypolimnas bolina*. J. Insect Sci. **11**: 92.

Mock, A., K. Tajovský, M. Žurovcová, A. Jarošová, P. Kocourek, J. Gruber, D. Angyal, and J. Spelda, 2016 *Hungarosoma bokori* Verhoeff, 1928 (Diplopoda: Chordeumatida): new insights into its taxonomy, systematics, molecular genetics, biogeography and ecology. Zootaxa **4178**: 234–256.

Molloy, J. C. and S. P. Sinkins, 2015 *Wolbachia* do not induce reactive oxygen species-dependent immune pathway activation in *Aedes albopictus*. Viruses **7**: 4624–4639.

Monaghan, P., N. B. Metcalfe, and R. Torres, 2009 Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol. Lett. **12**: 75–92.

Moné, Y., D. Monnin, and N. Kremer, 2014 The oxidative environment: a mediator of interspecies communication that drives symbiosis evolution. Proc. R. Soc. B **281**: 20133112.

Moran, N. A. and D. B. Sloan, 2015 The hologenome concept: helpful or hollow? PLoS Biol. **13**: e1002311.

Moran, N. A. and J. J. Wernegreen, 2000 Lifestyle evolution in symbiotic bacteria: insights from genomics. Trends Ecol. Evol. **15**: 321–326.

Moreira, L. A., I. Iturbe-Ormaetxe, J. A. Jeffery, G. Lu, A. T. Pyke, L. M. Hedges, *et al.*, 2009 A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and *Plasmodium*. Cell **139**: 1268–1278.

Morrow, J. L., M. Frommer, D. C. A. Shearman, and M. Riegler, 2014 Tropical tephritid fruit fly community with high incidence of shared *Wolbachia* strains as platform for horizontal transmission of endosymbionts. Environ. Microbiol. **16**: 3622–3637.

Mouchiroud, L., R. H. Houtkopper, N. Moullan, E. Katsyuba, D. Ryu, C. Cantó, *et al.*, 2013 The NAD+/sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. Cell **154**: 430–441.

Mousson, L., E. Martin, K. Zouache, Y. Madec, P. Mavingui, and A.-B. Failloux, 2010 *Wolbachia* modulates Chikungunya replication in *Aedes albopictus*. Mol. Ecol. **19**: 1953–1964.

Mousson, L., K. Zouache, C. Arias-Goeta, v. Raquin, P. Mavingui, and A.-B. Failloux, 2012 The native *Wolbachia* symbionts limit transmission of Dengue virus in *Aedes albopictus*. PLoS Negl. Trop. Dis. **6**: e1989.

Nairz, M., D. Haschka, E. Demetz, and G. Weiss, 2014 Iron at the interface of immunity and infection. Front. Pharmacol. **5**: 152.

Nappi, A. J. and E. Vass, 2002 Interactions of iron with reactive intermediates of oxygen and nitrogen. Dev. Neurosci. **24**: 134–142.

Narita, S., D. Kageyama, M. Nomura, and T. Fukatsu, 2007 Unexpected mechanism of symbiont-induced reversal of insect sex: feminizing *Wolbachia* continuously acts on the butterfly *Eurema hecabe* during larval development. Appl. Environ. Microbiol. **73**: 4332–4341.

Negri, I., 2012 *Wolbachia* as an "infectious" extrinsic factor manipulating host signaling pathways. Front. Endocrinol. **2**: 115.

Negri, I., A. Franchini, E. Gonella, D. Daffonchio, P. J. Mazzoglio, M. Mandrioli, and A. Alma, 2009 Unravelling the *Wolbachia* evolutionary role: the reprogramming of the host genomic imprinting. Proc. R. Soc. B **276**: 2485–2491.

Newton, I. L. G., O. Savytskyy, and K. B. Sheehan, 2015 *Wolbachia* utilize host actin for efficient maternal transmission in *Drosophila melanogaster*. PLoS Pathog. **11**: e1004798.

Nor, I., J. Engelstädter, O. Duron, M. Reuter, M.-F. Sagot, and S. Charlat, 2013 On the genetic architecture of cytoplasmic incompatibility: inference from phenotypic data. Am. Nat. **182**: E15–E24.

Normark, B. B., 2004 Haplodiploidy as an outcome of coevolution between male-killing cytoplasmic elements and their hosts. Evolution **58**: 790–798.

Oliveira, J. H. M., R. L. S. Gonçalves, F. A. Lara, F. A. Dias, A. C. P. Gandara, R. F. S. Menna-Barreto, *et al.*, 2011 Blood meal-derived heme decreases ROS levels in the midgut of *Aedes aegypti* and allows proliferation of intestinal microbiota. PLoS Pathog. **7**: e1001320.

Oliveira, M. F., J. R. Silva, M. Dansa-Petretski, W. de Souza, U. Lins, C. M. S. Braga, *et al.*, 1999 Haem detoxification by an insect. Nature **400**: 517–518.

O'Neill, S. L., R. Giordano, A. M. E. Colbert, T. L. Karr, and H. M. Robertson, 1992 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc. Natl. Acad. Sci. U.S.A. **89**: 2699–2702.

Osborne, S. E., I. Iturbe-Ormaetxe, J. C. Brownlie, S. L. O'Neill, and K. N. Johnson, 2012 Antiviral protection and the importance of *Wolbachia* density and tissue tropism in *Drosophila simulans*. Appl. Environ. Microbiol. **78**: 6922–6929.

Osborne, S. E., Y. S. Leong, S. L. O'Neill, and K. N. Johnson, 2009 Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. PLoS Pathogens **5**: e1000656.

Ote, M., M. Ueyama, and D. Yamamoto, 2016 *Wolbachia* protein TomO targets nanos mRNA and restores germ stem cells in *Drosophila Sex-lethal* mutants. Curr. Biol. **26**: 2223–2232.

Paiva-Silva, G. O., C. Cruz-Oliveira, E. S. Nakayasu, C. M. Maya-Monteiro, B. C. Dunkov, H. Masuda, *et al.*, 2006 A heme-degradation pathway in a blood-sucking insect. Proc. Natl. Acad. Sci. U.S.A. **103**: 8030–8035.

Palmer, W. J. and F. M. Jiggins, 2015 Comparative genomics reveals the origins and diversity of arthropod immune systems. Mol. Biol. Evol. **32**: 2111–2129.

Pan, X., G. Zhou, J. Wu, G. Bian, P. Lu, A. S. Raikhel, and Z. Xi, 2012 *Wolbachia* induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control dengue virus in the mosquito *Aedes aegypti*. Proc. Natl. Acad. Sci. U.S.A. **109**: E23–E31.

Pannebakker, B. A., B. Loppin, C. P. H. Elemans, L. Humblot, and F. Vavre, 2007 Parasitic inhibition of cell death facilitates symbiosis. Proc. Natl. Acad. Sci. U.S.A. **104**: 213–215.

Pannebakker, B. A., L. P. Pijnacker, B. J. Zwaan, and L. W. Beukeboom, 2004 Cytology of *Wolbachia*-induced parthenogenesis in *Leptopilina clavipes* (Hymenoptera: Figitidae). Genome **47**: 299–303.

Pannebakker, B. A., N. S. Schidlo, G. J. F. Boskamp, L. Dekker, T. J. M. van Dooren, L. W. Beukeboom, B. J. Zwaan, P. M. Brakefield, and J. J. M. van Alphen, 2005 Sexual functionality of *Leptopilina clavipes* (Hymenoptera: Figitidae) after reversing *Wolbachia*-induced parthenogenesis. J. Evol. Biol. **18**: 1019–1028.

Panteleev, D. Y., I. I. Goryacheva, B. V. Andrianov, N. L. Reznik, O. E. Lazebny, and A. M. Kulikov, 2007 The endosymbiotic bacterium *Wolbachia* enhances the nonspecific resistance to insect pathogens and alters behavior of *Drosophila melanogaster*. Russ. J. Genet. **43**: 1066–1069.

Paredes, J. C., D. P. Welchman, M. Poidevin, and B. Lemaitre, 2011 Negative regulation by amidase PGRPs shapes the *Drosophila* antibacterial response and protects the fly from innocuous infection. Immunity **35**: 770–779.

Parratt, S. R., C. L. Frost, M. A. Schenkel, A. Rice, G. D. D. Hurst, and K. C. King, 2016 Superparasitism drives heritable symbiont epidemiology and host sex ratio in a wasp. PLoS Pathog. **12**: e1005629.

Parsons, L. M., F. Lin, and J. Orban, 2006 Peptidoglycan recognition by Pal, an outer membrane lipoprotein. Biochemistry **45**: 2122–2128.

Perrot-Minnot, M.-J., B. Cheval, A. Migeon, and M. Navajas, 2002 Contrasting effects of *Wolbachia* on cytoplasmic incompatibility and

fecundity in the haplodiploid mite *Tetranychus urticae*. J. Evol. Biol. **15**: 808–817.

Pichon, S., D. Bouchon, R. Cordaux, L. Chen, R. A. Garrett, and P. Grève, 2009 Conservation of the type IV secretion system throughout *Wolbachia* evolution. Biochem. Biophys. Res. Commun. **385**: 557–562.

Pietri, J. E., H. DeBruhl, and W. Sullivan, 2016 The rich somatic life of *Wolbachia*. MicrobiologyOpen **5**: 923–936.

Pigeault, R., C. Braquart-Varnier, I. Marcadé, G. Mappa, E. Mottin, and M. Sicard, 2014 Modulation of host immunity and reproduction by horizontally acquired *Wolbachia*. J. Insect Physiol. **70**: 125–133.

Pijls, J. W. A. M., H. J. van Steenbergen, and J. J. M. van Alphen, 1996 Asexuality cured: the relations and differences between sexual and asexual *Apoanagyrus diversicornis*. Heredity **76**: 506–513.

Pike, N. and R. Kingcombe, 2009 Antibiotic treatment leads to the elimination of *Wolbachia* endosymbionts and sterility in the diplodiploid collembolan *Folsomia candida*. BMC Biol. **7**: 54.

Poinsot, D., S. Charlat, and H. Merçot, 2003 On the mechanism of *Wolbachia*-induced cytoplasmic incompatibility: confronting the models with the facts. Bioessays **25**: 259–265.

Pontier, S. M. and F. Schweisguth, 2015 A *Wolbachia*-sensitive communication between male and female pupae controls gamete compatibility in *Drosophila*. Curr. Biol. **25**: 2339–2348.

Prakash, A. and A. Monteiro, 2016 Molecular mechanisms of secondary sexual trait development in insects. Curr. Opin. Insect Sci. **17**: 40–48.

Prout, T., 1994 Some evolutionary possibilities for a microbe that causes incompatibility in its host. Evolution **48**: 909–911.

Puttaraju, H. P. and B. M. Prakash, 2009 Effects of elimination of *Wolbachia* on oogenesis of the uzifly *Exorista sorbillans*, a parasitoid of the silkworm *Bombyx mori*. Entomol. Res. **39**: 372–379.

Queller, D. C. and J. E. Strassmann, 2009 Beyond society: the evolution of organismality. Phil. Trans. R. Soc. B **364**: 3143–3155.

Queller, D. C. and J. E. Strassmann, 2016 Problems of multi-species organisms: endosymbionts to holobionts. Biol. Philos. **31**: 855–873.

Raha, S. and B. H. Robinson, 2000 Mitochondria, oxygen free radicals, disease and ageing. Trends Biochem. Sci. **25**: 502–508.

Rainey, S. M., J. Martinez, M. McFarlane, P. Juneja, P. Sarkies, A. Lulla, E. Schnettler, *et al.*, 2016 *Wolbachia* blocks viral genome replication early in infection without a transcriptional response by the endosymbiont or host small RNA pathways. PLoS Pathog. **12**: e1005536.

Rainey, S. M., P. Shah, A. Kohl, and I. Dietrich, 2014 Understanding the *Wolbachia*-mediated inhibition of arboviruses in mosquitoes: progress and challenges. J. Gen. Virol. **95**: 517–530.

Rancès, E., T. K. Johnson, J. Popovici, I. Iturbe-Ormaetxe, T. Zakir, C. G. Warr, and S. L. O'Neill, 2013 The Toll and Imd pathways are not required for *Wolbachia* mediated dengue virus interference. J. Virol. **87**: 11945–11949.

Rancès, E., Y. H. Ye, M. Woolfit, E. A. McGraw, and S. L. O'Neill, 2012 The relative importance of innate immune priming in *Wolbachia*-mediated dengue interference. PLoS Pathogens **8**: e1002548.

Randerson, J. P., N. G. Smith, and L. D. Hurst, 2000 The evolutionary dynamics of male-killers and their hosts. Heredity **84**: 152–160.

Rasgon, J. L., C. E. Gamston, and X. Ren, 2006 Survival of *Wolbachia pipientis* in cell-free medium. Appl. Environ. Microbiol. **72**: 6934–6937.

Rasmussen, S. A., D. J. Jamieson, M. A. Honein, and L. R. Petersen, 2016 Zika virus and birth defects — reviewing the evidence for causality. N. Engl. J. Med. **374**: 1981–1987.

Raychoudhury, R., L. Baldo, D. C. Oliveira, and J. H. Werren, 2009 Modes of acquisition of *Wolbachia*: horizontal transfer, hybrid introgression, and codivergence in the *Nasonia* species complex. Evolution **63**: 165–183.

Raychoudhury, R. and J. H. Werren, 2012 Host genotype changes bidirectional to unidirectional cytoplasmic incompatibility in *Nasonia longicornis*. Heredity **108**: 105–114.

Regier, J. C., J. W. Shultz, A. Zwick, A. Hussey, B. Ball, R. Wetzer, J. W. Martin, and C. W. Cunningham, 2010 Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. Nature **463**: 1079–1083.

Reynolds, K. T. and A. A. Hoffmann, 2002 Male age, host effects and the weak expression or non-expression of cytoplasmic incompatibility in *Drosophila* strains infected by maternally transmitted *Wolbachia*. Genet. Res. **80**: 79–87.

Richardson, K. M., M. Schiffer, P. C. Griffin, S. F. Lee, and A. A. Hoffmann, 2016 Tropical *Drosophila pandora* carry *Wolbachia* infections causing cytoplasmic incompatibility or male killing. Evolution **70**: 1791–1802.

Riegler, M., S. Charlat, C. Stauffer, and H. Merçot, 2004 *Wolbachia* transfer from *Rhagoletis cerasi* to *Drosophila simulans*: investigating the outcomes of host-symbiont coevolution. Appl. Environ. Microbiol. **70**: 273–279.

Rigaud, T. and P. Juchault, 1992 Genetic control of the vertical transmission of a cytoplasmic sex factor in *Armadillidium vulgare* Latr. (Crustacea, Oniscidea). Heredity **68**: 47–52.

Rigaud, T. and P. Juchault, 1995 Success and failure of horizontal transfers of feminizing *Wolbachia* endosymbionts in woodlice. J. Evol. Biol. **8**: 249–255.

Rigaud, T., P. S. Pennings, and P. Juchault, 2001 *Wolbachia* bacteria effects after experimental interspecific transfers in terrestrial isopods. J. Invertebr. Pathol. **77**: 251–257.

Riparbelli, M. G., R. Giordano, M. Ueyama, and G. Callaini, 2012 *Wolbachia*-mediated male killing is associated with defective chromatin remodeling. PLoS ONE **7**: e30045.

Ristow, M. and K. Schmeisser, 2014 Mitohormesis: promoting health and lifespan by increased levels of reactive oxygen species (ROS). Dose Resp. **12**: 288–341.

Robert, C. A. M., D. L. Frank, K. A. Leach, T. C. J. Turlings, B. E. Hibbard, and M. Erb, 2013 Direct and indirect plant defenses are not suppressed by endosymbionts of a specialist root herbivore. J. Chem. Ecol. **39**: 507–515.

Rodriguero, M. S., V. A. Confalonieri, J. V. C. Guedes, and A. A. Lanteri, 2010 *Wolbachia* infection in the tribe Naupactini (Coleoptera, Curculionidae): association between thelytokous parthenogenesis and infection status. Insect Mol. Biol. **19**: 631–640.

Rokas, A., R. J. Atkinson, J. L. Nieves-Aldrey, S. A. West, and G. N. Stone, 2002 The incidence and diversity of *Wolbachia* in gallwasps (Hymenoptera; Cynipidae) on oak. Mol. Ecol. **11**: 1815–1829.

Ros, V. I. D., V. M. Fleming, E. J. Feil, and J. A. J. Breeuwer, 2012 Diversity and recombination in *Wolbachia* and *Cardinium* from *Bryobia* spider mites. BMC Microbiol. **12** (Suppl. 1): S13.

Rossi, P., I. Ricci, A. Cappelli, C. Damiani, U. Ulissi, M. V. Mancini, M. Valzano, *et al.*, 2015 Mutual exclusion of *Asaia* and *Wolbachia* in the reproductive organs of mosquito vectors. Parasit. Vectors **8**: 278.

Roth, O., B. M. Sadd, P. Schmid-Hempel, and J. Kurtz, 2009 Strain-specific priming of resistance in the red flour beetle, *Tribolium castaneum*. Proc. R. Soc. B **276**: 145–151.

Rottschaefer, S. M. and B. P. Lazzaro, 2012 No effect of *Wolbachia* on resistance to intracellular infection by pathogenic bacteria in *Drosophila* melanogaster. PLoS ONE **7**: e40500.

Rousset, F., D. Bouchon, B. Pintureau, P. Juchault, and M. Solignac, 1992 *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. Proc. R. Soc. Lond. B **250**: 91–98.

Rousset, F., M. Raymond, and F. Kjellberg, 1991 Cytoplasmic incompatibilities in the mosquito *Culex pipiens*: How to explain a cytotype polymorphism? J. Evol. Biol. **4**: 69–81.

Roxström-Lindquist, K., O. Terenius, and I. Faye, 2004 Parasite-specific immune response in adult *Drosophila melanogaster*: a genomic study. EMBO Reports **5**: 207–212.

Roy, B. A. and J. W. Kirchner, 2000 Evolutionary dynamics of pathogen resistance and tolerance. Evolution **54**: 51–63.

Russell, J. A., B. Goldman-Huertas, C. Moreau, L. Baldo, J. K. Stahlhut, J. H. Werren, and N. E. Pierce, 2009 Specialization and

geographic isolation among *Wolbachia* symbionts from ants and lycaenid butterflies. Evolution **63**: 624–640.

Russell, J. E. and R. Stouthamer, 2011 The genetics and evolution of obligate reproductive parasitism in *Trichogramma pretiosum* infected with parthenogenesis-inducing *Wolbachia*. Heredity **106**: 58–67.

Ryu, J.-H., E.-M. Ha, C.-T. Oh, J.-H. Seol, P. T. Brey, I. Jin, *et al.*, 2006 An essential complementary role of NF-κB pathway to microbicidal oxidants in *Drosophila* gut immunity. EMBO J. **25**: 3693–3701.

Sabin, L. R., S. L. Hanna, and S. Cherry, 2010 Innate antiviral immunity in *Drosophila*. Curr. Opin. Immunol. **22**: 4–9.

Sacchi, L., M. Genchi, E. Clementi, I. Negri, A. Alma, S. Ohler, D. Sassera, K. Bourtzis, and C. Bandi, 2010 Bacteriocyte-like cells harbour *Wolbachia* in the ovary of *Drosophila melanogaster* (Insecta, Diptera) and *Zyginidia pullula* (Insecta, Hemiptera). Tissue Cell **42**: 328–333.

Sachs, J. L., C. J. Essenberg, and M. M. Turcotte, 2011a New paradigms for the evolution of beneficial infections. Trends Ecol. Evol. **26**: 202–209.

Sachs, J. L., U. G. Mueller, T. P. Wilcox, and J. J. Bull, 2004 The evolution of cooperation. Q. Rev. Biol. **79**: 135–160.

Sachs, J. L. and E. L. Simms, 2006 Pathways to mutualism breakdown. Trends Ecol. Evol. **21**: 585–592.

Sachs, J. L., R. G. Skophammer, and J. U. Regus, 2011b Evolutionary transitions in bacterial symbiosis. Proc. Natl. Acad. Sci. U.S.A. **108** (Suppl. 2): 10800–10807.

Sakamoto, H., D. Kageyama, S. Hoshizaki, and Y. Ishikawa, 2007 Sex-specific death in the Asian corn borer moth (*Ostrinia furnacalis*) infected with *Wolbachia* occurs across larval development. Genome **50**: 645–652.

Salunke, B. K., R. C. Salunkhe, D. P. Dhotre, S. A. Walujkar, A. B. Khandagale, R. Chaudhari, R. K. Chandode, *et al.*, 2012 Determination of *Wolbachia* diversity in butterflies from Western Ghats, India, by a multigene approach. Appl. Environ. Microbiol. **78**: 4458–4467.

Sánchez, L., 2008 Sex-determining mechanisms in insects. Int. J. Dev. Biol. **52**: 837–856.

Sansonetti, P. J. and R. Medzhitov, 2009 Learning tolerance while fighting ignorance. Cell **138**: 416–420.

Sazama, E. J., M. J. Bosch, C. S. Shouldis, S. P. Ouellette, and J. S. Wesner, 2017 Incidence of *Wolbachia* in aquatic insects. Ecol. Evol. **7**: 1165–1169.

Scherz-Shouval, R. and Z. Elazar, 2011 Regulation of autophagy by ROS: physiology and pathology. Trends Biochem. Sci. **36**: 30–38.

Schilthuizen, M. and R. Stouthamer, 1997 Horizontal transmission of parthenogenesis-inducing microbes in *Trichogramma* wasps. Proc. R. Soc. Lond. B **264**: 361–366.

Schmid-Hempel, P., 2003 Variation in immune defence as a question of evolutionary ecology. Proc. R. Soc. B **270**: 357–366.

Schneider, D. S. and J. S. Ayres, 2008 Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. Nat. Rev. Immunol. **8**: 889–895.

Schulenburg, H., J. Kurtz, Y. Moret, and M. T. Siva-Jothy, 2009 Introduction. Ecological immunology. Phil. Trans. R. Soc. B **364**: 3–14.

Schuler, H., C. Bertheau, S. P. Egan, J. L. Feder, M. Riegler, B. C. Schlick-Steiner, *et al.*, 2013 Evidence for a recent horizontal transmission and spatial spread of *Wolbachia* from endemic *Rhagoletis cerasi* (Diptera: Tephritidae) to invasive *Rhagoletis cingulata* in Europe. Mol. Ecol. **22**: 4101–4111.

Schulz, T. J., K. Zarse, A. Voigt, N. Urban, M. Birringer, and M. Ristow, 2007 Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab. **6**: 280–293.

Schwenke, R. A., B. P. Lazzaro, and M. F. Wolfner, 2016 Reproduction–immunity trade-offs in insects. Annu. Rev. Entomol. **61**: 239–256.

Scotti, P. D., S. Dearing, and D. W. Mossop, 1983 Flock House virus: a nodavirus isolated from *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae). Arch. Virol. **75**: 181–189.

Segoli, M., R. Stouthamer, C. M. Stouthamer, P. Rugman-Jones, and J. A. Rosenheim, 2013 The effect of *Wolbachia* on the lifetime reproductive success of its insect host in the field. J. Evol. Biol. **26**: 2716–2720.

Sena, L. A. and N. S. Chandel, 2012 Physiological roles of mitochondrial reactive oxygen species. Mol. Cell **48**: 158–167.

Serbus, L. R., C. Casper-Lindley, F. Landmann, and W. Sullivan, 2008 The genetics and cell biology of *Wolbachia*-host interactions. Annu. Rev. Genet. **42**: 683–707.

Serbus, L. R. and W. Sullivan, 2007 A cellular basis for *Wolbachia* recruitment to the host germline. PLoS Pathog. **3**: e190.

Shaw, D. K., X. Wang, L. J. Brown, A. S. Oliva Chávez, K. E. Reif, A. A. Smith, A. J. Scott, *et al.*, 2017 Infection-derived lipids elicit an immune deficiency circuit in arthropods. Nat. Commun. **8**: 14401.

Sheehan, K. B., M. Martin, C. F. Lesser, R. R. Isberg, and I. L. G. Newton, 2016 Identification and characterization of a candidate *Wolbachia pipientis* type IV effector that interacts with the actin cytoskeleton. mBio **7**: e00622–16.

Sheeley, S. L. and B. F. McAllister, 2009 Mobile male-killer: similar *Wolbachia* strains kill males of divergent *Drosophila* hosts. Heredity **102**: 286–292.

Sheldon, B. C. and S. Verhulst, 1996 Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol. Evol. **11**: 317–321.

Silva, I. M. M. S., 1999 *Identification and evaluation of Trichogramma parasitoids for biological pest control*. Ph.D. thesis, Wageningen University, The Netherlands.

Silva, I. M. M. S. and R. Stouthamer, 1997 To mate or not to mate... Can sex pheromones be used as a taxonomic tool in *Trichogramma* spp.? Proc. Exp. Appl. Entomol. **8**: 41–46.

Simões, P. M., G. Mialdea, D. Reiss, M.-F. Sagot, and S. Charlat, 2011 *Wolbachia* detection: an assessment of standard PCR protocols. Mol. Ecol. Resour. **11**: 567–572.

Simmonds, H. W., 1923 All female families of *Hypolimnas bolina* L., bred in Fiji by H. W. Simmonds. Proc. R. Entomol. Soc. Lond. pp. ix–xii.

Simon, H.-U., A. Haj-Yehia, and F. Levi-Schaffer, 2000 Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis **5**: 415–418.

Sinkins, S. P., 2013 *Wolbachia* and arbovirus inhibition in mosquitoes. Future Microbiol. **8**: 1249–1256.

Sintupachee, S., J. R. Milne, S. Poonchaisri, V. Baimai, and P. Kittayapong, 2006 Closely related *Wolbachia* strains within the pumpkin arthropod community and the potential for horizontal transmission via the plant. Microb. Ecol. **51**: 294–301.

Siozios, S., P. Sapountzis, P. Ioannidis, and K. Bourtzis, 2008 *Wolbachia* symbiosis and insect immune response. Insect Sci. **15**: 89–100.

Sommer, F. and F. Bäckhed, 2013 The gut microbiota — masters of host development and physiology. Nat. Rev. Microbiol. **11**: 227–238.

Son, Y., S. Luckhart, X. Zhang, M. J. Lieber, and E. E. Lewis, 2008 Effects and implications of antibiotic treatment on *Wolbachia*-infected vine weevil (Coleoptera: Curculionidae). Agr. Forest Entomol. **10**: 147–155.

Sontowski, R., D. Bernhard, C. Bleidorn, M. Schlegel, and M. Gerth, 2015 *Wolbachia* distribution in selected beetle taxa characterized by PCR screens and MLST data. Ecol. Evol. **5**: 4345–4353.

Souto-Maior, C., J. S. Lopes, E. Gjini, C. J. Struchiner, L. Teixeira, and M. G. M. Gomes, 2015 Heterogeneity in symbiotic effects facilitates *Wolbachia* establishment in insect populations. Theor. Ecol. **8**: 53–65.

Speakman, J. R. and M. Garratt, 2014 Oxidative stress as a cost of reproduction: beyond the simplistic trade-off model. Bioessays **36**: 93–106.

Speakman, J. R. and C. Selman, 2011 The free-radical damage theory: accumulating evidence against a simple link of oxidative stress to ageing and lifespan. Bioessays **33**: 255–259.

Stahlhut, J. K., C. A. Desjardins, M. E. Clark, L. Baldo, J. A. Russell, J. H. Werren, and J. Jaenike, 2010 The mushroom habitat

as an ecological arena for global exchange of *Wolbachia*. Mol. Ecol. **19**: 1940–1952.

Starr, D. J. and T. W. Cline, 2002 A host-parasite interaction rescues *Drosophila* oogenesis defects. Nature **418**: 76–79.

Stearns, S. C., 1989 Trade-offs in life-history evolution. Funct. Ecol. **3**: 259–268.

Stolk, C. and R. Stouthamer, 1996 Influence of a cytoplasmic incompatibility-inducing *Wolbachia* on the fitness of the parasitoid wasp *Nasonia vitripennis*. Proc. Exp. Appl. Entomol. **7**: 33–37.

Stork, N. E., J. McBroom, C. Gely, and A. J. Hamilton, 2015 New approaches narrow global species estimates for beetles, insects, and terrestrial arthropods. Proc. Natl. Acad. Sci. U.S.A. **112**: 7519–7523.

Stouthamer, R. and D. J. Kazmer, 1994 Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. Heredity **73**: 317–327.

Stouthamer, R., R. F. Luck, and W. D. Hamilton, 1990 Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex. Proc. Natl. Acad. Sci. U.S.A. **87**: 2424–2427.

Stouthamer, R., J. E. Russell, F. Vavre, and L. Nunney, 2010 Intragenomic conflict in populations infected by parthenogenesis inducing *Wolbachia* ends with irreversible loss of sexual reproduction. BMC Evol. Biol. **10**: 229.

Stuart, J. A., L. A. Maddalena, M. Merilovich, and E. L. Robb, 2014 A midlife crisis for the mitochondrial free radical theory of aging. Longev. Healthspan **3**: 4.

Sugimoto, T. N., T. Fujii, T. Kayukawa, H. Sakamoto, and Y. Ishikawa, 2010 Expression of a *doublesex* homologue is altered in sexual mosaics of *Ostrinia scapulalis* moths infected with *Wolbachia*. Insect Biochem. Mol. Biol. **40**: 847–854.

Sugimoto, T. N. and Y. Ishikawa, 2012 A male-killing *Wolbachia* carries a feminizing factor and is associated with degradation of the sex-determining system of its host. Biol. Lett. **8**: 412–415.

Sugimoto, T. N., T. Kayukawa, T. Shinoda, Y. Ishikawa, and T. Tsuchida, 2015 Misdirection of dosage compensation underlies bidirectional sex-specific death in Wolbachia-infected *Ostrinia scapulalis*. Insect Biochem. Mol. Biol. **66**: 72–76.

Suh, Y.-A., R. S. Arnold, B. Lassegue, J. Shi, X. Xu, D. Sorescu, *et al.*, 1999 Cell transformation by the superoxide-generating oxidase Mox1. Nature **401**: 79–82.

Sun, X., L. Cui, and Z. Li, 2007 Diversity and phylogeny of *Wolbachia* infecting *Bactrocera dorsalis* (Diptera: Tephritidae) populations from China. Environ. Entomol. **36**: 1283–1289.

Teixeira, L., A. Ferreira, and M. Ashburner, 2008 The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. PLoS Biol. **6**: e1000002.

Terradas, G., D. A. Joubert, and E. A. McGraw, 2017 The RNAi pathway plays a small part in *Wolbachia*-mediated blocking of dengue virus in mosquito cells. Sci. Rep. **7**: 43847.

Thomas-Orillard, M., 1990 Paradoxical influence of an RNA virus on *Drosophila* host population. Endocyt. Cell Res. **7**: 97–104.

Thomas-Orillard, M., B. Jeune, and G. Cusset, 1995 *Drosophila*-host genetic control of susceptibility to *Drosophila* C virus. Genetics **140**: 1289–1295.

Timmermans, M. J. T. N. and J. Ellers, 2009 *Wolbachia* endosymbiont is essential for egg hatching in a parthenogenetic arthropod. Evol. Ecol. **23**: 931–942.

Toivonen, J. M., G. A. Walker, P. Martinez-Diaz, I. Bjedov, Y. Driege, H. T. Jacobs, D. Gems, and L. Partridge, 2007 No influence of *Indy* on lifespan in *Drosophila* after correction for genetic and cytoplasmic background effects. PLoS Genet. **3**: e95.

Toomey, M. E., K. Panaram, E. M. Fast, C. Beatty, and H. M. Frydman, 2013 Evolutionarily conserved *Wolbachia*-encoded factors control pattern of stem-cell niche tropism in *Drosophila* ovaries and favor infection. Proc. Natl. Acad. Sci. U.S.A. **110**: 10788–10793.

Tortosa, P., S. Charlat, P. Labbé, J.-S. Dehecq, H. Barré, and M. Weill, 2010 *Wolbachia* age-sex-specific density in *Aedes albopictus*: a host evolutionary response to cytoplasmic incompatibility? PLoS ONE **5**: e9700.

Tram, U., P. M. Ferree, and W. Sullivan, 2003 Identification of *Wolbachia*–host interacting factors through cytological analysis. Microbes Infect. **5**: 999–1011.

Tsai, K.-H., C.-G. Huang, W.-J. Wu, C.-K. Chuang, C.-C. Lin, and W.-J. Chen, 2006 Parallel infection of Japanese encephalitis virus and *Wolbachia* within cells of mosquito salivary glands. J. Med. Entomol. **43**: 752–756.

Turelli, M., 1994 Evolution of incompatibility-inducing microbes and their hosts. Evolution **48**: 1500–1513.

Turelli, M. and N. H. Barton, 2017 Deploying dengue-suppressing *Wolbachia*: robust models predict slow but effective spatial spread in *Aedes aegypti*. Theor. Popul. Biol. **115**: 45–60.

Tzou, P., S. Ohresser, D. Ferrandon, M. Capovilla, J. M. Reichhart, B. Lemaitre, *et al.*, 2000 Tissue-specific inducible expression of antimicrobial peptide genes in *Drosophila* surface epithelia. Immunity **13**: 737–748.

Úbeda, F. and B. B. Normark, 2006 Male killers and the origins of paternal genome elimination. Theor. Popul. Biol. **70**: 511–526.

Unckless, R. L., 2011 A DNA virus of *Drosophila*. PLoS ONE **6**: e26564.

Unckless, R. L. and J. Jaenike, 2012 Maintenance of a male-killing *Wolbachia* in *Drosophila innubila* by male-killing dependent and male-killing independent mechanisms. Evolution **66**: 678–689.

Vala, F., T. Van Opijnen, J. A. J. Breeuwer, and M. W. Sabelis, 2003 Genetic conflicts over sex ratio: mite-endosymbiont interactions. Am. Nat. **161**: 254–266.

Van den Hurk, A. F., S. Hall-Mendelin, A. T. Pyke, F. D. Frentiu, K. McElroy, A. Day, S. Higgs, and S. L. O'Neill, 2012 Impact of *Wolbachia* on infection with Chikungunya and yellow fever viruses in the mosquito vector *Aedes aegypti*. PLoS Negl. Trop. Dis. **6**: e1892.

Van Meer, M. M. and R. Stouthamer, 1999 Cross-order transfer of *Wolbachia* from *Muscidifurax uniraptor* (Hymenoptera: Pteromalidae) to *Drosophila simulans* (Diptera: Drosophilidae). Heredity **82**: 163–169.

Vandel, A., 1941 Recherches sur la génétique et la sexualité des isopodes terrestres. VI: Les phénomènes de monogénie chez les oniscoïdes. Bull. biol. de la France et de Belgique **75**: 316–363.

Vanthournout, B. and F. Hendrickx, 2016 Hidden suppression of sex ratio distortion suggests Red queen dynamics between *Wolbachia* and its dwarf spider host. J. Evol. Biol. **29**: 1488–1494.

Vautrin, E., S. Charles, S. Genieys, and F. Vavre, 2007 Evolution and invasion dynamics of multiple infections with *Wolbachia* investigated using matrix based models. J. Theor. Biol. **245**: 197–209.

Vautrin, E., S. Genieys, S. Charles, and F. Vavre, 2008 Do vertically transmitted symbionts co-existing in a single host compete or cooperate? A modelling approach. J. Evol. Biol. **21**: 145–161.

Vavre, F. and S. Charlat, 2012 Making (good) use of *Wolbachia*: what the models say. Curr. Opin. Microbiol. **15**: 263–268.

Vavre, F., P. Fouillet, and F. Fleury, 1999a Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. Mol. Biol. Evol. **16**: 1711–1723.

Vavre, F., P. Fouillet, and F. Fleury, 2003 Between- and within-host species selection on cytoplasmic incompatibility-inducing *Wolbachia* in haplodiploids. Evolution **57**: 421–427.

Vavre, F., C. Girin, and M. Boulétreau, 1999b Phylogenetic status of a fecundity-enhancing *Wolbachia* that does not induce thelytoky in *Trichogramma*. Insect Mol. Biol. **8**: 67–72.

Vavre, F., N. Kremer, B. A. Pannebakker, B. Loppin, and P. Mavingui, 2008 Is symbiosis evolution influenced by the pleiotropic role of programmed cell death in immunity and development? In *Insect Symbiosis,* Vol. 3, edited by K. Bourtzis and T. A. Miller, pp. 57–76, CRC Press, Boca Raton.

Veneti, Z., M. E. Clark, T. L. Karr, C. Savakis, and K. Bourtzis, 2004 Heads or tails: host-parasite interactions in the *Drosophila–Wolbachia* system. Appl. Environ. Microbiol. **70**: 5366–5372.

Veneti, Z., S. Zabalou, G. Papafotiou, C. Paraskevopoulos, S. Pattas, I. Livadaras, G. Markakis, *et al.*, 2012 Loss of reproductive parasitism following transfer of male-killing *Wolbachia* to *Drosophila melanogaster* and *Drosophila simulans*. Heredity **109**: 306–312.

Vollmer, J., A. Schiefer, T. Schneider, K. Jülicher, K. L. Johnston, M. J. Taylor, *et al.*, 2013 Requirement of lipid II biosynthesis for cell division in cell wall-less *Wolbachia*, endobacteria of arthropods and filarial nematodes. Int. J. Med. Microbiol. **303**: 140–149.

Voronin, D., D. A. N. Cook, A. Steven, and M. J. Taylor, 2012 Autophagy regulates *Wolbachia* populations across diverse symbiotic associations. Proc. Natl. Acad. Sci. U.S.A. **109**: E1638–E1646.

Voronin, D., A. F. Guimarães, G. R. Molyneux, K. L. Johnston, L. Ford, and M. J. Taylor, 2014 *Wolbachia* lipoproteins: abundance, localisation and serology of *Wolbachia* peptidoglycan associated lipoprotein and the Type IV Secretion System component, VirB6 from *Brugia malayi* and *Aedes albopictus*. Parasit. Vectors **7**: 462.

Wade, M. J. and N. W. Chang, 1995 Increased male fertility in *Tribolium confusum* beetles after infection with the intracellular parasite *Wolbachia*. Nature **373**: 72–74.

Walker, T., P. H. Johnson, L. A. Moreira, I. Iturbe-Ormaetxe, F. D. Frentiu, C. J. McMeniman, Y. S. Leong, *et al.*, 2011 The *w*Mel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature **476**: 450–453.

Wang, L., C. Zhou, Z. He, Z.-G. Wang, J.-L. Wang, and Y.-F. Wang, 2012 *Wolbachia* infection decreased the resistance of *Drosophila* to lead. PLoS ONE **7**: e32643.

Wang, Z. and M. Wu, 2015 An integrated phylogenomic approach toward pinpointing the origin of mitochondria. Sci. Rep. **5**: 7949.

Waterhouse, R. M., E. V. Kriventseva, S. Meister, Z. Xi, K. S. Alvarez, L. C. Bartholomay, C. Barillas-Mury, *et al.*, 2007 Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes. Science **316**: 1738–1743.

Watts, D. J. and S. H. Strogatz, 1998 Collective dynamics of 'small-world' networks. Nature **393**: 440–442.

Weeks, A. R. and J. A. J. Breeuwer, 2001 *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. Proc. R. Soc. Lond. B **268**: 2245–2251.

Weeks, A. R., M. Turelli, W. R. Harcombe, K. T. Reynolds, and A. A. Hoffmann, 2007 From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. PLoS Biol. **5**: e114.

Weinert, L. A., E. V. Araujo-Jnr, M. Z. Ahmed, and J. J. Welch, 2015 The incidence of bacterial endosymbionts in terrestrial arthropods. Proc. R. Soc. B **282**: 20150249.

Weinert, L. A., M. C. Tinsley, M. Temperley, and F. M. Jiggins, 2007 Are we underestimating the diversity and incidence of insect bacterial symbionts? A case study in ladybird beetles. Biol. Lett. **3**: 678–681.

Weinert, L. A., J. H. Werren, A. Aebi, G. N. Stone, and F. M. Jiggins, 2009 Evolution and diversity of *Rickettsia* bacteria. BMC Biol. **7**: 6.

Werren, J. H., 1987 The coevolution of autosomal and cytoplasmic sex ratio factors. J. Theor. Biol. **124**: 317–334.

Werren, J. H., 1997 Biology of *Wolbachia*. Annu. Rev. Entomol. **42**: 587–609.

Werren, J. H., 2011 Selfish genetic elements, genetic conflict, and evolutionary innovation. Proc. Natl. Acad. Sci. U.S.A. **108** (Suppl. 2): 10863–10870.

Werren, J. H., L. Baldo, and M. E. Clark, 2008 *Wolbachia*: master manipulators of invertebrate biology. Nat. Rev. Microbiol. **6**: 741–751.

Werren, J. H. and L. W. Beukeboom, 1998 Sex determination, sex ratios, and genetic conflict. Annu. Rev. Ecol. Syst. **29**: 233–261.

Werren, J. H. and S. L. O'Neill, 1997 The evolution of heritable symbionts. In *Influential Passengers. Inherited Microorganisms and Arthropod Reproduction*, edited by S. L. O'Neill, A. A. Hoffmann, and J. H. Werren, pp. 1–41, Oxford University Press, Oxford, UK.

Werren, J. H., D. Windsor, and L. R. Guo, 1995a Distribution of *Wolbachia* among neotropical arthropods. Proc. R. Soc. Lond. B **262**: 197–204.

Werren, J. H. and D. M. Windsor, 2000 *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? Proc. R. Soc. Lond. B **267**: 1277–1285.

Werren, J. H., W. Zhang, and L. R. Guo, 1995b Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. Proc. R. Soc. Lond. B **261**: 55–63.

West, S. A., R. M. Fisher, A. Gardner, and E. T. Kiers, 2015 Major evolutionary transitions in individuality. Proc. Natl. Acad. Sci. U.S.A. **112**: 10112–10119.

White, P. M., J. E. Pietri, A. Debec, S. Russell, B. Patel, and W. Sullivan, 2017a Mechanisms of horizontal cell-to-cell transfer of *Wolbachia* spp. in *Drosophila melanogaster*. Appl. Environ. Microbiol. **83**: e03425–16.

White, P. M., L. R. Serbus, A. Debec, A. Codina, W. Bray, A. Guichet, R. S. Lokey, and W. Sullivan, 2017b Reliance of *Wolbachia* on high rates of host proteolysis revealed by a genome-wide RNAi screen of *Drosophila* cells. Genetics **205**: 1473–1488.

Williams, T. A. and T. M. Embley, 2015 Changing ideas about eukaryotic origins. Phil. Trans. R. Soc. B **370**: 20140318.

Wiwatanaratanabutr, I., 2013 Distribution, diversity and density of wolbachial infections in cladocerans and copepods from Thailand. J. Invertebr. Pathol. **114**: 341–345.

Wiwatanaratanabutr, I., P. Kittayapong, Y. Caubet, and D. Bouchon, 2009 Molecular phylogeny of *Wolbachia* strains in arthropod hosts based on *groE*-homologous gene sequences. Zool. Sci. **26**: 171–177.

Wong, Z. S., J. C. Brownlie, and K. N. Johnson, 2015 Oxidative stress correlates with *Wolbachia*-mediated antiviral protection in *Wolbachia-Drosophila* associations. Appl. Environ. Microbiol. **81**: 3001–3005.

Wong, Z. S., L. M. Hedges, J. C. Brownlie, and K. N. Johnson, 2011 *Wolbachia*-mediated antibacterial protection and immune gene regulation in *Drosophila*. PLoS ONE **6**: e25430.

Wu, S.-C., C.-W. Liao, R.-L. Pan, and J.-L. Juang, 2012 Infection-induced intestinal oxidative stress triggers organ-to-organ immunological communication in *Drosophila*. Cell Host Microbe **11**: 410–417.

Xi, Z., L. Gavotte, Y. Xie, and S. L. Dobson, 2008 Genome-wide analysis of the interaction between the endosymbiotic bacterium *Wolbachia* and its *Drosophila* host. BMC Genomics **9**: 1.

Xue, X., S.-J. Li, M. Z. Ahmed, P. J. De Barro, S.-X. Ren, and B.-L. Qiu, 2012 Inactivation of *Wolbachia* reveals its biological roles in whitefly host. PLoS ONE **7**: e48148.

Yakob, L. and T. Walker, 2016 Zika virus outbreak in the Americas: the need for novel mosquito control methods. Lancet Glob. Health **4**: e148–e149.

Yamamura, N., 1993 Vertical transmission and evolution of mutualism from parasitism. Theor. Pop. Biol. **44**: 95–109.

Yang, X.-H., D.-H. Zhu, Z. Liu, L. Zhao, and C.-Y. Su, 2013 High levels of multiple infections, recombination and horizontal transmission of *Wolbachia* in the *Andricus mukaigawae* (Hymenoptera; Cynipidae) communities. PLoS ONE **8**: e78970.

Yano, T., S. Mita, H. Ohmori, Y. Oshima, Y. Fujimoto, R. Ueda, *et al.*, 2008 Autophagic control of *Listeria* through intracellular innate immune recognition in *Drosophila*. Nat. Immunol. **9**: 908–916.

Ye, Y. H., M. Woolfit, E. Rancès, S. L. O'Neill, and E. A. McGraw, 2013 *Wolbachia*-associated bacterial protection in the mosquito *Aedes aegypti*. PLoS Negl. Trop. Dis. **7**: e2362.

You, H., W. J. Lee, and W.-J. Lee, 2014 Homeostasis between gut-associated microorganisms and the immune system in *Drosophila*. Curr. Opin. Immunol. **30**: 48–53.

Yun, J. and T. Finkel, 2014 Mitohormesis. Cell Metab. **19**: 757–766.

Yun, Y., Y. Peng, F. X. Liu, and C. Lei, 2011 *Wolbachia* screening in spiders and assessment of horizontal transmission between predator and prey. Neotrop. Entomol. **40**: 164–169.

Zarse, K., S. Schmeisser, M. Groth, S. Priebe, G. Beuster, D. Kuhlow, *et al.*, 2012 Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. Cell Metab. **15**: 451–465.

Zchori-Fein, E., C. Borad, and A. R. Harari, 2006 Oogenesis in the date stone beetle, *Coccotrypes dactyliperda*, depends on symbiotic bacteria. Physiol. Entomol. **31**: 164–169.

Zchori-Fein, E. and K. Bourtzis, editors, 2011 *Manipulative Tenants: Bacteria Associated with Arthropods*. CRC Press, Boca Raton.

Zchori-Fein, E., O. Faktor, M. Zeidan, Y. Gottlieb, H. Czosnek, and D. Rosen, 1995 Parthenogenesis-inducing microorganisms in *Aphytis* (Hymenoptera: Aphelinidae). Insect Mol. Biol. **4**: 173–178.

Zchori-Fein, E., R. T. Roush, and M. S. Hunter, 1992 Male production induced by antibiotic treatment in *Encarsia formosa* (Hymenoptera: Aphelinidae), an asexual species. Experientia **48**: 102–105.

Zchori-Fein, E., R. T. Roush, and D. Rosen, 1998 Distribution of parthenogenesis-inducing symbionts in ovaries and eggs of *Aphytis* (Hymenoptera: Aphelinidae). Curr. Microbiol. **36**: 1–8.

Zeh, J. A., M. M. Bonilla, A. J. Adrian, S. Mesfin, and D. W. Zeh, 2012 From father to son: transgenerational effect of tetracycline on sperm viability. Sci. Rep. **2**: 375.

Zélé, F., A. Nicot, A. Berthomieu, M. Weill, O. Duron, and A. Rivero, 2014 *Wolbachia* increases susceptibility to *Plasmodium* infection in a natural system. Proc. R. Soc. B **281**: 20132837.

Zélé, F., A. Nicot, O. Duron, and A. Rivero, 2012 Infection with *Wolbachia* protects mosquitoes against *Plasmodium*-induced mortality in a natural system. J. Evol. Biol. **25**: 1243–1252.

Zhang, H., K.-J. Zhang, and X.-Y. Hong, 2010 Population dynamics of noncytoplasmic incompatibility-inducing *Wolbachia* in *Nilaparvata lugens* and its effects on host adult life span and female fitness. Environ. Entomol. **39**: 1801–1809.

Zhang, L., N. C. Ging, T. Komoda, T. Hanada, T. Suzuki, and K. Watanabe, 2005 Antibiotic susceptibility of mammalian mitochondrial translation. FEBS Letters **579**: 6423–6427.

Zhang, Y.-K., X.-L. Ding, X. Rong, and X.-Y. Hong, 2015 How do hosts react to endosymbionts? A new insight into the molecular mechanisms underlying the *Wolbachia*-host association. Insect Mol. Biol. **24**: 1–12.

Zhao, D.-X., D.-S. Chen, C. Ge, T. Gotoh, and X.-Y. Hong, 2013a Multiple infections with *Cardinium* and two strains of *Wolbachia* in the spider mite *Tetranychus phaselus* Ehara: Revealing new forces driving the spread of *Wolbachia*. PLoS ONE **8**: e54964.

Zhao, D.-X., X.-F. Zhang, and X.-Y. Hong, 2013b Host-symbiont interactions in spider mite *Tetranychus truncates* doubly infected with *Wolbachia* and *Cardinium*. Environ. Entomol. **42**: 445–452.

Zheng, Y., P.-P. Ren, J.-L. Wang, and Y.-F. Wang, 2011a *Wolbachia*-induced cytoplasmic incompatibility is associated with decreased *Hira* expression in male *Drosophila*. PLoS ONE **6**: e19512.

Zheng, Y., J.-L. Wang, C. Liu, C.-P. Wang, T. Walker, and Y.-F. Wang, 2011b Differentially expressed profiles in the larval testes of *Wolbachia* infected and uninfected *Drosophila*. BMC Genomics **12**: 595.

Zhou, W., F. Rousset, and S. O'Neill, 1998 Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. Proc. R. Soc. Lond. B **265**: 509–515.

Zilber-Rosenberg, I. and E. Rosenberg, 2008 Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiol. Rev. **32**: 723–735.

Zouache, K., D. Voronin, V. Tran-Van, L. Mousson, A.-B. Failloux, and P. Mavingui, 2009 Persistent *Wolbachia* and cultivable bacteria infection in the reproductive and somatic tissues of the mosquito vector *Aedes albopictus*. PLoS ONE **4**: e6388.

Zuk, M. and A. M. Stoehr, 2015 Immune defense and host life history. Am. Nat. **160**: S9–S22.

Publications

Zug, R. and P. Hammerstein, 2012 Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS ONE **7**: e38544.

Zug, R., A. Koehncke, and P. Hammerstein, 2012 Epidemiology in evolutionary time: the case of *Wolbachia* horizontal transmission between arthropod host species. J. Evol. Biol. **25**: 2149–2160.

Zug, R. and P. Hammerstein, 2015a Bad guys turned nice? A critical assessment of *Wolbachia* mutualisms in arthropod hosts. Biol. Rev. **90**: 89–111.

Zug, R. and P. Hammerstein, 2015b *Wolbachia* and the insect immune system: what reactive oxygen species can tell us about the mechanisms of *Wolbachia*–host interactions. Front. Microbiol. **6**: 1201.

Gebiola, M., S. E. Kelly, L. Velten, R. Zug, P. Hammerstein, M. Giorgini, and M. S. Hunter, 2017 Reproductive interference and fecundity affect competitive interactions of sibling species with low mating barriers: experimental and theoretical evidence. Heredity **119**: 438–446.

Zug, R. and P. Hammerstein, 2018 Evolution of reproductive parasites with direct fitness benefits. Heredity **120**: 266–281.

Acknowledgements

First and foremost, I would like to thank Peter Hammerstein for his supervision and his great support throughout the years. Above all, I thank Peter for his generosity and his trust, giving me the freedom to pursue the questions that I deemed interesting. I have benefited immensely from his ability to keep an eye on the big picture while acknowledging that even small changes can make all the difference. Not least, I really appreciate the friendly relationship we have.

I am grateful to Adam Wilkins for encouraging the writing of what is now Chapter 4 of this thesis, and for being a continuous source of advice. Moreover, I would like to thank Jack Werren for much valuable input. His suggestions are present throughout the thesis and have greatly improved it. This thesis also benefited from discussions with and stimulations from Jan Engelstädter, Daisuke Kageyama, and Arndt Telschow—thank you. Special thanks go to Arnulf Köhncke: he not only contributed significantly to what is now Chapter 3, but was also of great help in many other ways.

Furthermore, I'd like to thank my past and present colleagues at the ITB for many great conversations and activities, both in and out of science—Victor Anaya, Grisha Bordyugov, Benjamin Bossan, Sofia Figueiredo, Matthias Flor, Andreas Hantschmann, Kirsten Hilgenböcker, Tobias Kaufholz, Sarah Lück, Martina Michalikova, Agnes Rosahl, Pia Rose, Robert Schmidt, Lennart Velten, Franziska Witzel, Wei Wu, and many more.

I am very thankful to Andreas Hantschmann, Elvira Lauterbach, Rike-Benjamin Schuppner, Karin Winklhöfer, and Tiziano Zito for their great administrative and technical support. I also gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft (SFB 618).

After all, life happens mostly outside the office. I thank my friends for their time, support, and commitment. Together, we've had the best of times, and it ain't over yet. Thank you, Sebastian, for our special friendship. It means a lot to me.

I am so happy to be with you, Lina. You give me so much. Thank you for our common journey.

Finally, I thank my family for their unconditional love. I wouldn't be who I am without you.

Selbstständigkeitserklärung

Ich erkläre, dass ich die vorliegende Arbeit selbstständig und nur unter Verwendung der angegebenen Literatur und Hilfsmittel angefertigt habe.

Berlin, den Roman Zug